Vol. XLVI No. 9

September 1957

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

CONTENTS OF THIS ISSUE

Effect of Alterations in Side Chain upon Anti-inflammatory and Liver Glycogen Activities of Hydrocortisone Esters..... 515
Charles A. Winter and *Curt C. Porter*

Surfactants in Tablets to Improve Disintegration..... 520
Ben F. Cooper and *E. A. Brecht*

An Investigation of the Incompatibilities of Quarternary Ammonium Germicides in Compressed Troches I. The Adsorption of Cetylpyridinium Chloride and Benzalkonium Chloride by Talc and Kaolin..... 524
Nicholas H. Batuyios and *E. A. Brecht*

Stability of Atropine in Aqueous Solution..... 531
Albert A. Kondritzer and *Peter Zvirblis*

Enzymatic Decomposition of Digitalis Glycosides..... 535
Ole Gisvold and *S. E. Wright*

Antimicrobial Properties of a Series of β-Piperidinopropiones and Related Derivatives..... 539
H. J. Florestano, M. E. Bahler, and *A. D. Chandler*

Alkoxy Derivatives of β-Piperidinopropiophenone and Related Compounds..... 542
Earl R. Bockstahler and *Donald L. Wright*

A Comparative Human *In Vivo* Study of Antacids..... 545
E. W. Packman, D. D. Abbott, J. I. Feinman, and *Joseph W. E. Harrisson*

Comparative *In Vivo* Methods for Evaluating Antacids in Humans..... 549
Joseph W. E. Harrisson, D. D. Abbott, J. L. Feinman, and *E. W. Packman*

A Now Accurate and Reliable Method to Record Blood Coagulation Time Using and A. C. Bridge Principle..... 553
Alfred W. Richardson and *Jack G. Bishop*

An Investigation of the Possible Potentiation of the Analgetic Properties of Morphine by Mephenesin..... 556
Sumner M. Robinson and *Howard J. Jenkins*

A Method for the Measurement of Lubricating Property of Medicinal Products..... 558
Gerhard Levy and *T. W. Schwarz*

Viscosity Changes of Sodium Alginate Solutions after Freezing and Thawing..... 562
T. W. Schwarz and *Gerhard Levy*

Studies with a Uterine Antispasmodic, Ambucetamide..... 564
J. B. Hoekstra, Dorothea S. Fisher, K. M. Cull, D. E. Tisch, and *H. L. Dickison*

A Note on the Effect of Boric Acid and Sodium Metaborate on the Oxygen Uptake of Brain Tissue..... 569
Alfred S. C. Ling, Edward B. Truitt, Jr., and *John C. Krantz, Jr.*

A Note on Crystallographic and X-Ray Diffraction Data for Ethinamate..... 571
Ann Van Camp and *R. R. Pfeiffer*

A Note on Mathematics of Oral Sustained Release Products..... 572
Eino Nelson

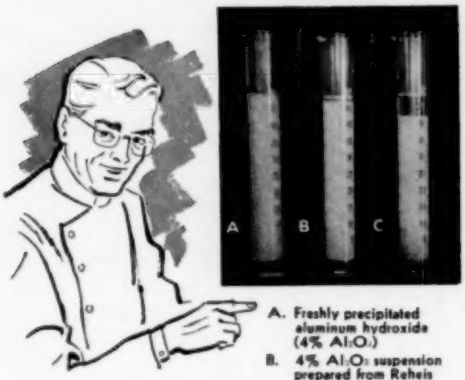
Books Received..... 573

Book Notices..... 574

Advertising.....Cover pages, 2, 3, 4, and I to IV

© *Copyright 1957, by the American Pharmaceutical Association*

just what the doctor ordered . .



A. Freshly precipitated aluminum hydroxide (4% Al_2O_3)
B. 4% Al_2O_3 suspension prepared from Reheis F-1000
C. 4% Al_2O_3 suspension prepared from Reheis F-2000

REHEIS

$Al(OH)_3$ GELS

Medical authorities have long recognized that an aqueous suspension of freshly precipitated Aluminum Hydroxide is the best form in which to prescribe this medication. Since this is not always practical, especially for the ambulatory patient, the physician must then revert to tablets, capsules, or powders.

Reheis Dried Aluminum Hydroxide Gels are so prepared that they retain all the desirable properties of the freshly precipitated gel. *Their hydrophilic properties are not destroyed and, as demonstrated above, can be used whenever a liquid product is desired.*

Be sure to give the doctor what he wants in the product he prescribes, be it liquid, tablet, or capsule.

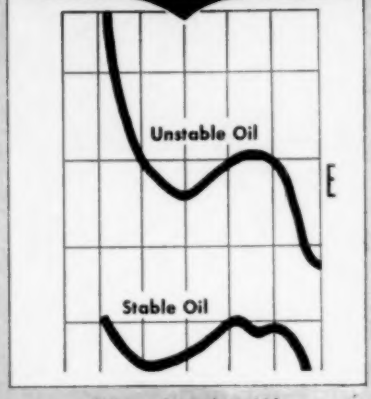
Write today on your letterhead for free bulletin and samples.

Take advantage of the services of our Technical Service Department if you have any problems.

1346

REHEIS COMPANY, INC.
Manufacturers of Fine Chemicals
BERKELEY HEIGHTS · NEW JERSEY

Penn-Drake® SPECTROPHOTOMETRIC CONTROL



Wave length—AU

Now Assures White Oils of HIGHEST STABILITY

The ultraviolet absorption spectrum gives us a reliable quantitative measure of minute impurities in white oils—even as little as a few parts per million—which heretofore defied detection during ordinary analysis. With this tool, Penn-Drake controls the refining process scientifically to eliminate objectionable substances. Uniform, dependable light stability and purity are thus determined quickly with a degree of certainty unattainable before.

Perhaps our experience in this field can help you solve a product or process problem. Our Technical Service Department will be glad to consult with you.

penn drake products

Pennsylvania Refining Company
BUTLER 23, PENNSYLVANIA
Branches: Cleveland, Ohio and Edgewater, N.J.
Representatives in all Principal Cities

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

SCIENTIFIC EDITION

Committee on Publications

John B. Heinz, *Chairman*
John J. Dugan
Robert P. Fischelis
Glenn L. Jenkins
Hugo H. Schaefer

Editorial Advisory Board

John E. Christian
Walter C. Gakenheimer
George P. Hager
Ewart A. Swinyard
Elmer B. Vliet
H. W. Youngken, Jr.

JUSTIN L. POWERS, EDITOR
SAMUEL W. GOLDSTEIN, ASSSISTANT EDITOR
ROBERT I. BISCHOFF, ADVERTISING MANAGER

The Massachusetts College of Pharmacy
Longwood Avenue, Boston

Graduate study in Boston has always appealed strongly to students in the health professions. The environment is stimulating and attracts students from all parts of the world. Library, laboratory, hospital, and other facilities for graduate work are outstanding in this area.

The Massachusetts College of Pharmacy is located in this great educational center. It offers unusual opportunities to graduates of colleges of pharmacy who are interested in further study in pharmacy, pharmaceutical chemistry, and pharmacognosy.

For additional information, write to HOWARD C. NEWTON, Dean

The *Journal of the American Pharmaceutical Association* is published monthly by the American Pharmaceutical Association in two editions: *Scientific Edition* and *Practical Pharmacy Edition*, under the supervision of the Committee on Publications of the Council of the Association. Publication Office: 20th and Northampton Streets, Easton, Pennsylvania. Executive, Editorial, and Advertising Offices: 2215 Constitution Avenue, N. W., Washington 7, D. C. **Annual Subscription**—United States, Canada, Pan American, and Spain: *Practical Pharmacy Edition* $5.00, *Scientific Edition* $6.00, both editions $10.00. Other foreign: *Practical Pharmacy Edition* $6.00, *Scientific Edition* $7.00, both editions $12.00. Subscription rates are subject to change without notice. Members of the American Pharmaceutical Association receive the combined subscription as part of their annual dues. Members may request omission of either edition, but this will not reduce the amount of the annual dues. **Single Copies**—*Practical Pharmacy Edition*: United States, Canada, Pan America, and Spain $0.50; other foreign $0.60. *Scientific Edition:* United States, Canada, Pan America, and Spain $0.60; other foreign $0.70. **Change of Address**—Members and subscribers should notify both the Post Office and the American Pharmaceutical Association about any change of address immediately. **Claims**—Missing numbers will not be supplied if claims are received more than 60 days after the date of issue, or if loss was due to failure to give notice of change of address. The Association cannot accept responsibility for foreign delivery when its records indicate shipment has been made. **Entered** as second class matter January 23, 1917, at the Post Office at Easton, Pennsylvania, under the Act of March 3, 1879, as 24 times a year; *Scientific Edition* monthly on the 5th and *Practical Pharmacy Edition* monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

NOTICE TO AUTHORS OF PAPERS

Manuscripts.—All manuscripts submitted to the Editor or presented to the Sections of the ASSOCIATION should be ready for publication, typewritten with double spacing on one side of good quality paper 8.5″ x 11″ in size and with 1″ to 1.5″ margins. In addition to the original typewritten copy, one or more carbon copies should be submitted in order to facilitate examination by members of the Editorial Advisory Board or other referees. Each manuscript should be accompanied by an abstract of not more than 200 words which includes a summarization of conclusions and recommendations. All pages in manuscripts, including tables but not figures, should be numbered consecutively. Previously published and readily accessible procedures which have been followed in experimental work should not be made a part of the manuscript, but should only be referred to by appropriate literature references.

Form of Presentation.—Titles of manuscripts should be kept as short as possible and preferably restricted to one line. The name of the laboratory in which the work described was done should be placed in a footnote at the end of the first page. Each manuscript should begin with one or more introductory paragraphs indicating the intention and meaning of the investigation and the method of procedure. The introduction should be followed by a report of the essential details of the experimental work, and a discussion or interpretation where necessary. Finally there should be a brief summary of conclusions and recommendations. Authors may find it helpful to arrange manuscripts to conform to the style of presentation as illustrated in papers on analogous subjects published in recent issues of THIS JOURNAL.

In preparing manuscripts for publication in THIS JOURNAL, center headings should be avoided as far as possible. Side headings followed by a period and dash should be indented and run into the part of the text to which they apply. Such side headings may be used as freely as desired. Literature citations should be numbered consecutively in order of appearance in the manuscript and should be indicated by full-sized Arabic numerals enclosed in parentheses. If a literature reference is cited more than once in a manuscript, subsequent citations should be indicated by the first number assigned. References to footnotes throughout the text should be numbered consecutively by superscript Arabic numerals, but such references in each table should be designated by superscript lower case letters beginning with "a" and continuing in natural order. Literature citations should be grouped at the end of the manuscript under the heading "References." All bibliographic references should be checked with the original publications. The citations should be numbered consecutively with full-sized Arabic numerals enclosed in parentheses corresponding to the numbers used in the text. The names of all periodicals cited in the list of references must be abbreviated in accordance with abbreviations given by **Chemical Abstracts** in its "List of Periodicals Abstracted." The sequence followed in the citations must be as follows:

(1) Doe, J. B., *Am. J. Physiol.*, **79**, 289(1927).

References to books should be in the order given, (1) author's surname and initials, (2) title of the book, (3) volume, (4) edition, (5) name of publisher, (6) place of publication, (7) year of publication, and (8) page number. A complete book reference may be illustrated as follows:

(1) Gilman, H., "Organic Chemistry," Vol. 2, 2nd ed., John Wiley and Sons, New York, 1943, pp. 1236, 1252.

Arabic numerals must be used to designate all definite weights, measurements, percentages, and degrees of temperature unless occurring at the beginning of a sentence, in which case the numeral and measurement are spelled out. Periods of time should be written in words. Unusual characters and Greek letters should be written plainly and legibly or should be explained by notes on the margin of the page. Fractions should be written with a slant line. Standard abbreviations should be used whenever weights and measures are stated in the metric system, e. g., 10 Gm., 2.5 cc., etc. The forms to be used are: cc., Kg., Gm., mg., mm., cm., and L.

Figures.—All drawings should be made in India ink, preferably on white tracing paper or cloth. Original drawings are preferable, but clear, well-prepared glossy photographic prints are acceptable. The size of illustrations should not exceed the 8.5″ x 11″ manuscript size. They should be designed for reduction to the width of one column (2.75″) or full page width (5.5″). Thus, height rather than width should be accentuated where possible.

Numbers and measurements, etc., which are to appear on the axes of the drawing should be indicated in pencil outside the axes, in order that they may be set in type. Captions and legends should be placed *below* the diagram (not on it), or, if lengthy, collected on a separate typewritten list, identifying each by its proper figure number and including whatever acknowledgments are necessary.

Photographs should never be rolled, and paper clips should not be used unless guarded by a piece of paper folded several times to prevent an indentation in the photograph. All drawings and photographs should be protected from bending and from the chance of having their edges torn while in transit. Do not write on the back of a photograph. Any indentation in the photograph, however slight, will be likely to show as a blemish in the reproduction. All figures submitted must be referred to in the text of the manuscript, and should be numbered consecutively with Arabic numerals, e. g., Fig. 1, Fig. 2, etc. Kymograph tracings should not be submitted with the original experimental legends which are likely to have been hastily or carelessly inscribed. Authors may find it worth while to employ a professional draftsman to make the drawings in proper form for reproduction.

Tables.—Tables should be carefully constructed so that the data presented may be understood easily. The indiscriminate use of tables should be avoided. Tables should be constructed so as to occupy fully a single column (2.75″) which will take about 40–50 characters and spaces. Tables which require two columns are also acceptable. Interpretative statements should not be written on the body of a table but should be explained in footnotes. Column heads should be made as small as possible and standard abbreviations should be used liberally. Data which may easily be deduced by simple arithmetic from data in another column should not be included. All tables should be referred to in the text of the manuscript. Every table and every column should be supplied with an appropriate heading. It is not necessary to place tables on separate sheets, and in fact it is preferable to intersperse them in the manuscript. Tables should be numbered consecutively using Roman numerals. The table number and the title should be placed in a continuous heading above the data presented. The preparation of a clear and concise table of data requires much planning and infinite care.

CHEMICALLY Designed

FOR SUPERIOR PRODUCTS

Amerchols® are natural, non-ionic **OINTMENT BASES AND EMULSIFIERS** which contain *Lanolin Cholesterols in their Most Active Form.*

These stable cholesterol and multisterol surfactants induce rapid drug release, promote optimum healing rates, and are safe for the most delicate tissues. We know of no case of an allergy due to an **AMERCHOL.***

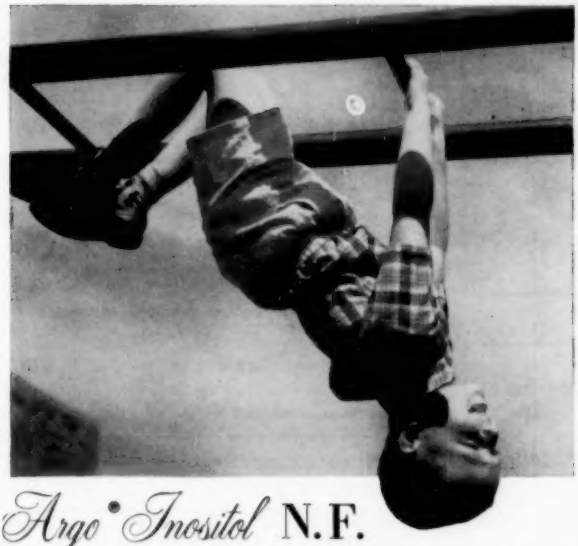
Modulan® is the acetyl derivative of pure lanolin† with unique new properties for use in **OINTMENTS AND EMULSIONS.** It imparts waxy, protective hydrophobic films; is oil soluble and compatible with O/W emulsions, soaps and shampoos. Clinical investigations indicate that **MODULAN IS HYPOALLERGENIC.***

† (U. S. PAT. NO. 2,725,334)

AMERICAN CHOLESTEROL PRODUCTS, INC. AMERCHOL

**References, technical data and suggested formulations are available from our research laboratories.*

American Cholesterol Products
• INCORPORATED •
AMERCHOL PARK EDISON, NEW JERSEY



Argo • Inositol N.F.

The lipotropic agent, INOSITOL N.F., a constituent of an important class of phospholipids, is a naturally-occurring component of the vitamin-B complex. Used alone or with certain vitamins this significant nutritional factor is effective in helping to regulate fat and cholesterol metabolism and, furthermore, is synergistic in its lipotropic action. INOSITOL N.F. is pure, crystalline, easy to handle and packaged in 5, 10, 50 and 100 pound containers for convenient use in formulations.

When you are considering new therapeutic formulations, investigate ARGO INOSITOL N.F. Information is constantly being received concerning this important factor in many pharmaceutical preparations and is supplied on request. In addition, our technical service staff will be pleased to work together with you on your formulation problems.

...Recommended for use in New Geriatric and Lipotropic Formulations

"Fine Chemicals from Corn"

CORN PRODUCTS SALES COMPANY

17 Battery Place, New York 4, N. Y.

FOOD RESEARCH LABORATORIES INC.
OUR 35th YEAR

RESEARCH
ANALYSES CONSULTATION

BIOLOGICAL, NUTRITIONAL AND TOXICOLOGICAL STUDIES FOR THE FOOD, DRUG AND ALLIED INDUSTRIES

48-14 33rd STREET, LONG ISLAND CITY 1, N.Y.
Western Office—9231 VENICE BOULEVARD, CULVER CITY, CALIF.

SINCE 1870

Analyses · Consultation · Research

New Drugs

Bio-assays · Toxicological Studies
Formula Development · Special Analyses
Litigation · Phenol Coefficients

LaWall & Harrisson
Div. P, 1921 Walnut St., Philadelphia 3, Pa.
Bacteriologists · Chemists · Pharmacologists

BOUND VOLUMES

Journal of the
American
Pharmaceutical
Association

$11.00

Order from the Association

2215 Constitution Avenue, N. W.
Washington 7, D. C.

DRUG STANDARDS

A Publication of
The American Pharmaceutical Association

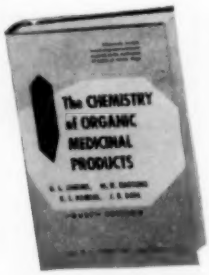
Single Copies $1.00
Annual Subscription $5.00

Order from

The American Pharmaceutical Association
2215 Constitution Avenue
Washington 7, D. C.

Ready Now!

The new *Fourth Edition* of



THE CHEMISTRY OF ORGANIC MEDICINAL PRODUCTS

By GLENN L. JENKINS, Purdue University; WALTER H. HARTUNG, Medical College of Virginia; KENNETH E. HAMLIN, Jr., Abbott Laboratories; and JOHN B. DATA, Purdue University.

Now completely revised to reflect the dramatic developments in recent years, this highly-regarded work continues to offer you a rational system of chemical classification for organic medicinal compounds, both natural and synthetic. It emphasizes the relationship between chemical structure and physical properties and physiological activity.

Note these important features:

- **Covers the most recent developments in the areas of the antibiotics, tranquilizing drugs, oral insulin products, antihistamines, and other medicinal agents.**
- **Stresses, where possible, the *in vivo* reaction and fate of medicinal compounds.**
- **Offers a broad, yet concise, coverage of the literature of organic pharmacy and medicinal chemistry, with key citations to more detailed discussions.**
- **Presents one of the most recent brief treatments of stereoisomerism.**

1957 **569 pages** **$10.75**

Mail this coupon TODAY for an ON-APPROVAL copy!

JOHN WILEY & SONS, Inc. JAPA-97
440 Fourth Ave., New York 16, N. Y.

Please send me a copy of THE CHEMISTRY OF ORGANIC MEDICINAL PRODUCTS to read and examine. Within 10 days I will return the book and owe nothing, or I will remit $10.75, plus postage.

Name.....

Address.....

City..... Zone..... State.....

( ) SAVE POSTAGE! Check here if you ENCLOSE payment, in which case we will pay the postage. Same return privilege, refund guaranteed.

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLVI — *SEPTEMBER 1957* — NUMBER 9

CONSECUTIVE NO. 18

Effect of Alterations in Side Chain upon Anti-inflammatory and Liver Glycogen Activities of Hydrocortisone Esters*

By CHARLES A. WINTER and CURT C. PORTER

A number of esters of hydrocortisone and related steroids have been examined for activity in the granuloma inhibition (subcutaneous cotton pellet) test, both after local application of the steroids and after systemic administration. Liver glycogen deposition activity was examined by a one-day oral test in mice, a six-day test in rats, and for duration of action after a single subcutaneous injection. Rates of absorption and activity per mg. of steroid absorbed also were determined. Wide differences in activity were found, depending upon the configuration at C-21. Part of this difference was due to diverse rates of absorption, but there were also differences in activity per mg. of steroid absorbed. It is concluded that ease of hydrolysis of the ester is also a factor in determining potency. The tertiary butyl acetate is particularly effective when locally applied, but systemic activity of this ester is relatively low because of reduced rate of absorption and decreased activity per mg. absorbed. After subcutaneous injection, the free alcohol of hydrocortisone is more rapidly absorbed and gives a more prompt response than any of its esters examined. The 9α-fluoro derivative is more gradually absorbed and gives a more sustained response than does hydrocortisone.

Since the discovery of the value of cortisone in the treatment of certain manifestations of collagen and other diseases, numerous analogs of the naturally occurring corticosteroids have been made and tested. Considerable emphasis has been placed on the effect upon biological potency of changes in the steroid nucleus and of changes in the oxygen functions. Most of these alterations lead to a reduction in activity, but some of them have given more active compounds than the naturally occurring steroids. Among the latter are the introduction of halide in the 9α-position (1, 2), of a double bond in position 1 (3), and of methyl on carbon 2 (4, 5).

Less attention has been paid to the effect upon biological activity of alterations in the side chain at carbon atom 21. It is not always appreciated that the results obtained in the assay of a given steroid may depend to a high degree upon rapidity of absorption from injection site or from the gastrointestinal tract, and that the rate of absorption may vary enormously depending upon the configuration of the side chain. Rate of hydrolysis of an ester may also be a factor. Porter and Silber (6) have shown that the acetate of hydrocortisone is less rapidly

* Received March 22, 1957, from the Merck Institute for Therapeutic Research, West Point, Pa.

This paper is dedicated to Professor Rolf Meier, on the occasion of his sixtieth birthday anniversary.

The steroids used in this study were prepared in the Merck Sharp & Dohme Research Laboratories by Dr. Karl Pfister III and Dr. L. H. Sarett and their colleagues. The authors are grateful for the technical assistance of Mrs. Elizabeth Gaffney, Miss Merle Greenwald, Dr. Seymour Winsten, and Mr. James Gilfillan.

absorbed and has less effect upon liver glycogen after subcutaneous or intramuscular injection than the free alcohol of the same compound. The present paper presents the results of our assays of anti-inflammatory activity and liver glycogen deposition of a number of esters of active steroids.

METHODS

The assay of anti-inflammatory activity used in this study was a modification of that described by Meier, Schuler, and Desaulles (7). Essentially it consisted of subcutaneous implantation of cotton pellets into rats, removal of the pellets after six or seven days, and obtaining the increment in dry weights as a measure of granuloma formation. The inhibiting effect of steroids was observed either after local application of the steroid to the pellet, or after subcutaneous injection at a site distant from the pellet, or after oral administration of steroid.

The pellets employed were 5-mm. sections cut from dental cotton rolls, weighing about 40 mg. They were sterilized in an autoclave, handled with sterile instruments and, as an additional precaution, antibiotics were added to the pellet. Two pellets were inserted in each animal, one on each side of the abdomen. When local effects of steroids were to be studied, a steroid-treated pellet was placed on one side and a pellet containing the suspending agent for the steroid on the other, so that each animal was its own control. Both pellets were treated with suspending agents when systemic effects of steroids were tested. Male Holtzman rats of 125- to 150-Gm. weight were employed. Intact animals in groups of five or six were used. Effects upon body-weight gain, adrenal weight, and thymus weight were determined at the same time. Results of the assay were calculated as per cent inhibition of dry weight of granuloma formation by the formula $100(A - B)/A$ where A = gain in mg. dry weight of control pellets, and B = gain in mg. dry weight of steroid-treated pellets (local test) or of pellets of steroid-treated animals (systemic test).

Three methods were employed in studying the effects of the esters upon the deposition of liver glycogen.

Method I.—Male albino rats, weighing about 200 Gm. each, were fed a protein-free diet for ten days and a 20%-casein diet for the subsequent three days. The steroids were administered daily by the subcutaneous route for the last six days of the experiment. The animals then were sacrificed and liver glycogen was determined as milligrams of reducing substances per 100 Gm. body weight.

Method II.—Male albino rats were given single subcutaneous injections of the steroids, and groups of animals were sacrificed at one, two, three, four, and seven days. Liver glycogen and residual steroids at the injection site were determined (6), and calculations were made of the area under the liver glycogen curve for overall activity and of activity per mg. of steroid absorbed.

Method III.—Albino mice weighing about 20 Gm. were fasted for eighteen hours and then were given oral doses of the steroids hourly for five doses. The animals were sacrificed one hour after the final dose, and liver glycogen was determined (8). Groups of five mice and two dose-levels of steroids were used.

RESULTS

In order to determine the optimal time for leaving the pellets in the animals, the experiment shown in Fig. 1 was performed. It is seen that either with, or without steroid, the gain in weight of the pellets was a linear function of time. Actually, this linearity continued for eight days and then leveled off. Six to seven days was chosen as a convenient duration of the experiments, because the difference between control and treated pellets was nearly maximal by that time, and also effects on adrenal and thymus weights were nearly maximal by then (9).

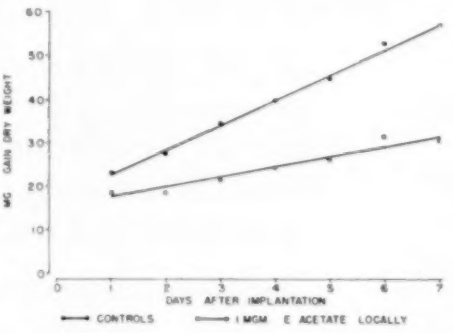


Fig. 1.—Regression lines of gain in dry weight of subcutaneously implanted cotton pellets. Upper: controls. Lower: pellets containing 1 mg. of cortisone acetate.

Figures 2 and 3 show dose-response curves for per cent inhibition of granuloma plotted against log dose of steroid. The data are calculated as parallel straight lines, and the fit is reasonably good for the data in the local experiments and for most of the systemic data. By means of the regression lines, relative potency can be calculated readily. It is at once apparent that potency varies greatly depending upon the structure of the side chain, and that the relative order of activity may be quite different when the compounds are compared by local application than when the same compounds are compared by subcutaneous administration. The less soluble esters often show the greatest activity in the local test and the least activity when systemically administered. When the compound is injected, it has to be absorbed and be carried to the site of action by the blood stream, but in the local test the compound may remain on the pellet to exert its action during the entire duration of the experiment. Thus, lack of solubility may be an advantage for local, but a disadvantage for systemic administration.

Particularly outstanding in potency in the local test are the tertiary butyl acetate (TBA) esters; prednisolone TBA is about 1000 times as active as cortisone in this test, but when administered subcutaneously it is no more potent than cortisone. Hydrocortisone TBA, about eight times as active as cortisone acetate in the local test, does not appear

TABLE I.—GRANULOMA INHIBITION ACTIVITIES BY THE COTTON-PELLET TEST OF ESTERS OF HYDROCORTISONE

Compound Number	C-21 Side Chain	Local Application		Subcutaneous Injection	
		Dose, γ	Granuloma Inhibition, %	Daily Dose, γ	Granuloma Inhibition, %
I	21-al-Dimethylacetal	1,000	0	...	..
II	Hemicamphorate	1,000	22	3,000	0
III	Caproate	1,000	35	2,000	54
		500	15		
		250	24	1,000	37
		125	5		
IV	Trimethylacetate	500	42	...	..
V	Hydrocinnamate	500	50	1,000	23
VI	Caprylate	500	50	...	..
VII	Mesylate	1,000	52	2,000	0
VIII	Diethylacetate	500	56	...	..
IX	Diphenylacetate	250	77	2,000	0
X	Tertiary butyl acetate	16	45	4,000	0
		8	34	8,000	11
		4	30		
		2	13		

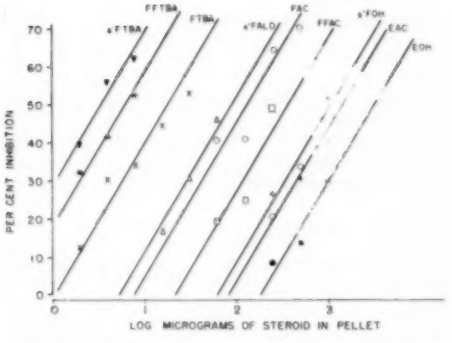


Fig. 2.—Regression lines of dose-response curves of granuloma inhibition by various derivatives of active steroids locally applied to the cotton pellets. From left to right, the steroids with the calculated amounts in micrograms to produce 50% inhibition are: prednisolone tertiary butyl acetate 3.5, 9α-fluorohydrocortisone tertiary butyl acetate 6.3, hydrocortisone tertiary butyl acetate 19, prednisolone-21-aldehyde 91, hydrocortisone acetate 140, 9α-fluorohydrocortisone acetate 370, prednisolone 1100, cortisone acetate 1500, cortisone 3400.

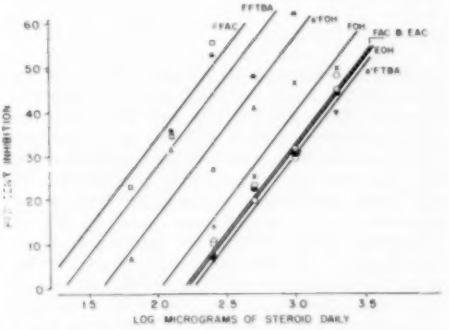


Fig. 3.—Regression lines of dose-response curves of granuloma inhibition by various derivatives of active steroids injected subcutaneously daily for seven days. From left to right, the steroids with calculated daily doses in mg. to produce 50% inhibition are: 9α-fluorohydrocortisone acetate 0.26, 9α-fluorohydrocortisone tertiary butyl acetate 0.35, prednisolone 0.69, hydrocortisone 1.8, hydrocortisone acetate and cortisone acetate 2.65, cortisone 2.85, prednisolone tertiary butyl acetate 3.25.

in Fig. 3 because it was nearly devoid of activity when injected subcutaneously. The data also show that substitution of TBA for acetate in 9α-fluorohydrocortisone acetate gives a compound more potent by the local test, but less potent by subcutaneous injection. Hydrocortisone (free alcohol) does not appear in Fig. 2 because in the local test the slope of the regression line was significantly less than that of any of those shown. Since this compound has relatively good solubility, a single application to the pellet may not have retained full effectiveness for the duration of the experiment.

The finding that cortisone acetate and hydrocortisone acetate have about the same activity in the systemic cotton-pellet test confirms the report of Singer and Borman (10). These authors did not give data on hydrocortisone (free alcohol), but our data show it to be about 1.5 times as active as hydrocortisone acetate by injection. The ratio of systemic activity of 9α-fluorohydrocortisone acetate to hydrocortisone acetate in our data is similar to that found by these authors.

The high degree of local activity of the TBA esters indicated that they might be useful for local therapy in rheumatic joints. Bilka (11), Hollander, *et al.* (12, 13), and Spies, *et al.* (14), reported hydrocortisone TBA to be more effective when administered intra-articularly than a variety of other steroids, including several other esters of hydrocortisone. Zuckner, *et al.* (15), on the other hand, found it effective but not certainly more effective than hydrocortisone.

Table I shows the results of screening of a few of the esters of hydrocortisone that we have examined. Although most of them were not available in sufficient quantities to obtain dose-response curves, it is apparent that there were great differences in potency. The first three compounds were

TABLE II.—LIVER GLYCOGEN ACTIVITY OF SOME HYDROCORTISONE ESTERS

Ester	Repletion Test: Dose, Molecular Equivalent of 1.5 mg. Hydrocortisone Acetate/100 Gm. Rat/Day, s.c. for 6 Days		Prolongation Test: Single s.c. Doses of the Molecular Equivalent of 8.25 mg. Hydrocortisone Acetate/100 Gm. Rat		
	Response[a]	Activity[b]	Prolongation[c] Activity (7 Days)	Absorbed in 7 Days, mg.	Activity[d] Wt. Absorbed
Free alcohol	285	=			
Acetate	370		347	1.3	267
Propionate	455	=			
Trimethylacetate	351	=	362	3.6	100
Diethylacetate	504	=			
t-Butyl acetate[e]	90	<	101	1.8	57
Caprylate	601	>			
Caproate[f]	643	>	737	5.5	135
Stearate	75	<	59	3.1	19
Benzoate	88	<	144	2.6	55
Phenylacetate	383	=	...	...	...
Cortisone acetate	389	=	...	...	...
Cortisone *t*-butyl acetate	136	<	...	...	...

[a] Milligrams liver-reducing substances per 100 Gm. body weight, above control values.
[b] Activity compared with hydrocortisone acetate by Student's *t* test.
[c] Area under curves relating liver-reducing substances to days on test.
[d] Calculated as hydrocortisone acetate.
[e] Hydrocortisone *t*-butyl acetate was about one-half as active as hydrocortisone alcohol in a one-day mouse liver glycogen test when administered orally (Method III).
[f] Hydrocortisone caproate was about as active as hydrocortisone alcohol in the mouse test (Method III).

no more than one-tenth as active as hydrocortisone acetate in the local test, and only the last one had appreciably greater local activity than the acetate. Compound X was about ten times as potent as the acetate in the local test. Most of the esters had little or no activity when given subcutaneously; only one of them, III, was as active as the free alcohol.

Therefore, it appears that the activity of hydrocortisone for local application may be greatly enhanced by variations in the side chain, but for systemic administration the free alcohol is the optimal configuration. Esters not listed in the table which were tested at single-dose levels locally, and showed a high degree of activity, but were not tested systemically include the phenylacetate, chloroacetate, stearate, benzoate, and palmitate.

The liver glycogen activities of a number of hydrocortisone esters, determined by Method I, are shown in the first two columns of Table II. Liver glycogen levels, as milligrams of total liver-reducing substances per 100 Gm. body weight, above control values are shown, and each ester is compared with hydrocortisone according to Student's *t* test. As in the cotton-pellet test, it is apparent that activity varies greatly among the various esters. The compounds were administered for six days, so that there was adequate time for absorption and cumulative effect, if any. The most active ester in the six-day test, the caproate, was no more active than the acetate in the one-day mouse test (Method III). The latter agrees with the results of the cotton-pellet test.

In the last three columns of Table II is shown the test for prolongation of action of the esters (Method II). It is interesting to note that the prolongation of activity after a single subcutaneous injection did not depend entirely upon rate of absorption. Less of the acetate was absorbed in seven days than any of the other esters tested, but its activity per mg. absorbed was far higher than any of the others. The caproate had the best overall activity because it was relatively well absorbed, although its activity, systemically per milligram absorbed, was only half that of the acetate.

The trimethylacetate was more readily absorbed than the acetate and had about the same overall activity. Desaulles and Meier (16) examined the trimethylacetate of cortisone, and found it to have prolonged duration of action compared to cortisone acetate. Porter and Silber (6) reported that cortisone and cortisone acetate were absorbed at about the same rate, but that hydrocortisone acetate was more persistent in its effect. Apparently, additional methyl groups do not prolong further the already long action of hydrocortisone acetate. It is probable that the esters have to be hydrolyzed by the body before exerting their effects, and the rate at which this occurs influences the overall potency as

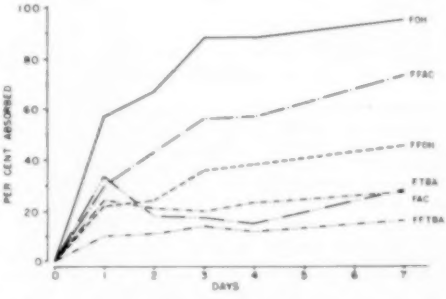


Fig. 4.—Rates of absorption of various steroids after single subcutaneous injection, determined by sacrifice of groups of five animals at various times. From top to bottom at Day 7: hydrocortisone, 9α-fluorohydrocortisone acetate, 9α-fluorohydrocortisone, hydrocortisone tertiary butyl acetate, hydrocortisone acetate, 9α-fluorohydrocortisone tertiary butyl acetate.

TABLE III.—LIVER GLYCOGEN ACTIVITY OF HYDROCORTISONE AND 9α-FLUOROHYDROCORTISONE AND THEIR ACETATES AND *t*-BUTYL ACETATES

Compound	Single Subcutaneous Doses of 5 mg. Hydrocortisone or Its Equivalent/100 Gm. Rat			Mouse Activity Oral Dose (Hydrocortisone = 1)
	Activity (7-Day Area)	Mg. Compound[a] Absorbed in 7 Days	Activity / mg. Absorbed	
Hydrocortisone	1,622	4.76	341	1
Hydrocortisone acetate	222	1.36	163	1
Hydrocortisone *t*-butyl acetate	65	0.33	197	0.5
Fluorohydrocortisone	2,385	2.27	1,050	4
Fluorohydrocortisone acetate	2,142	3.65	588	
Fluorohydrocortisone *t*-butyl acetate	937	0.81	1,155	2

[a] Calculated as hydrocortisone.

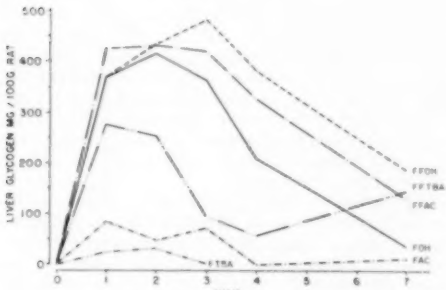


Fig. 5.—Liver glycogen values after single subcutaneous injection of various steroids. From top to bottom at Day 3: 9α-fluorohydrocortisone, 9α-fluorohydrocortisone acetate, hydrocortisone, 9α-fluorohydrocortisone tertiary butyl acetate, hydrocortisone acetate, hydrocortisone tertiary butyl acetate. Ordinates are calculated as mg. liver glycogen per 100 Gm. body weight above control values.

much as does rate of absorption. The relative difficulty of hydrolysis is reflected in the last column of Table II as activity per mg. of steroid absorbed.

In another experiment, the acetates and tertiary butyl acetates of hydrocortisone and 9α-fluorohydrocortisone were compared with the free alcohol forms by Method II after a single subcutaneous injection of 5 mg. of hydrocortisone or its molar equivalent of the other steroids. Figure 4 shows the rate of absorption as determined by sacrificing groups of animals at one, two, three, four, and seven days. Nearly 90% of hydrocortisone free alcohol was absorbed in three days. None of the other compounds approached this value, and only 16% of 9α-fluorohydrocortisone tertiary butyl acetate, and less than 30% of the hydrocortisone acetate or TBA was absorbed in seven days. It was surprising to find that the acetate of 9α-fluorohydrocortisone was better absorbed than the free alcohol throughout the period of observation.

Figure 5 shows the curves obtained for liver glycogen in this experiment. In contrast to the result obtained after daily injection where cumulative effects appear (Table I, column 1), the acetate of hydrocortisone has a relatively small effect on liver glycogen after a single injection. In keeping with its ready absorbability the effect of hydrocortisone free alcohol rises to a peak rapidly and falls to a low level at the end of the experiment. The effects of 9α-fluorohydrocortisone and 9α-fluorohydrocortisone acetate also rise to a peak rapidly, since these are inherently highly active compounds, and the level is better sustained than with the hydrocortisone alcohol, as they are more gradually absorbed.

Table III gives some of the pertinent data of this experiment. Large differences in total activity were noted; in each case, the fluoro derivative was considerably more potent than its corresponding hydrocortisone derivative. The overall potency of the TBA of 9α-fluorohydrocortisone was much less than that of the free alcohol, although the activity per mg. of steroid absorbed was about the same. This is in contrast to the TBA of hydrocortisone, which showed a reduction in activity per milligram absorbed compared to the free alcohol, although the hydrocortisone TBA had much higher activity per milligram in this experiment than in the previous test shown in Table II.

REFERENCES

(1) Fried, J., and Sabo, E. F., *J. Am. Chem. Soc.*, **75**, 2273(1953).
(2) Liddle, G. W., Pechet, M. M., and Bartter, F. C., *Science*, **120**, 496(1954).
(3) Herzog, H. L., Nobile, A., Tolksdorf, S., Charney, W., Hershberg, E. B., Perlman, P. L., and Pechet, M. M., *ibid.*, **122**, 176(1955).
(4) Hogg, J. A., Lincoln, F. H., Jackson, R. W., and Schneider, W. P., *J. Am. Chem. Soc.*, **77**, 6401(1955).
(5) Liddle, G. W., Richard, J. E., and Tomkins, G. M., *Metabolism*, **5**, 384(1956).
(6) Porter, C. C., and Silber, R. H., *Endocrinology*, **53**, 73(1953).
(7) Meier, R., Schuler, W., and Desaulles, P., *Experientia*, **6**, 469(1950).
(8) Venning, E. H., Kazmin, V. E., and Bell, J. C., *Endocrinology*, **38**, 79(1946).
(9) Winter, C. A., Silber, R. H., and Stoerk, H. C., *ibid.*, **47**, 60(1950).
(10) Singer, F. M., and Borman, A., *Federation Proc.*, **14**, 281(1955).
(11) Bilka, P. J., *Minnesota Med.*, **38**, 408(1955).
(12) Hollander, J. L., *Ann. N. Y. Acad. Sci.*, **61**, 511 (1955).
(13) Hollander, J. L., Brown, E. M., Jr., Jessar, R. A., Udell, L., Smukler, N. M., and Bowie, M. A., *J. Am. Med. Assoc.*, **158**, 476(1955).
(14) Spies, T. D., Stone, R. E., García-López, G., Díaz Tellechea, C. M., López Toca, R., Reboredo, A., and Suárez, R. M., *ibid.*, **159**, 645(1955).
(15) Zuckner, J., Machek, O., and Ahern, A. M., *Ann. Rheumatic Diseases*, **15**, 258(1956).
(16) Desaulles, P., and Meier, R., *Schweiz. med. Wochschr.*, **84**, 741(1954).

Surfactants in Tablets to Improve Disintegration*

By BEN F. COOPER† and E. A. BRECHT‡

Incorporation of surfactants into tablet formulations was effective in reducing disintegration times in most cases. Evaluation results of 21 surfactants are presented. The two most effective surfactants were Aerosol OT® and Aerosol MA®.

TABLET DISINTEGRATING agents generally fall into three classes: substances which absorb moisture and swell, effervescent substances which react with moisture to form a gas, and substances which react with moisture to reconstitute a foam. It has been suggested (1) that certain disintegrating agents may act by capillary action to draw moisture into the tablet. The greatest number of disintegrating agents which have been proposed have been of the type which absorb water and swell. Corn and potato starches are the most widely used of these swelling substances.

Evaluation of surfactants as disintegrating agents has not been reported in the literature. Surfactants have been evaluated as lubricants in compressed tablets (2). The purpose of this investigation was to study the effect on disintegration time when surfactants were incorporated into compressed tablet formulations.

EXPERIMENTAL

Tablet Evaluation Tests.—Each batch of tablets met the requirements of the U. S. P. Weight Variation Test. Tablet hardness was measured by means of the Monsanto Hardness Tester, and was recorded as the average of six tablets selected at random. Disintegration time was measured in accordance with U. S. P. directions, except that the time was recorded as the average of the first and last tablets to pass through the wire mesh. Preliminary experiments indicated that comparable results were obtained for artificial gastric juice and distilled water when used as disintegration media. Therefore, distilled water was used for the disintegration medium in all cases.

Tablet Formulations.—All tablets were compressed with a Stokes single punch machine. A summary of satisfactory formulations is presented in Table I.

Preliminary Test Tablets.—Preliminary tests were conducted to determine a suitable test tablet. A suitable tablet was considered one with a relatively long disintegration time, preferably containing an official drug as the active ingredient, and relatively stable under ordinary conditions. The following drugs were selected for evaluation as possible test tablets: acetylsalicylic acid; a combination of aspirin, phenacetin and caffeine; aluminum hydroxide, calcium lactate, sodium salicylate, sulfamerazine, and thyroid. Tablets were prepared with no disintegrating agent present and with 10% starch present. The disintegration times of calcium lactate and sodium salicylate tablets were exceptional (Table II).

The presence of starch appeared to hinder the disintegration of sodium salicylate tablets as shown by a longer disintegration time compared to similar tablets containing no disintegrating agent. It has been suggested (3) that starch forms a paste, enveloping soluble substances in such a way as to prevent ready solution of the tablet. This could not be substantiated for tablets containing the following soluble active ingredients: ammonium chloride, sodium bicarbonate, and sodium chloride.

In the case of calcium lactate tablets, the presence of starch appeared to have little or no effect on disintegration time. It was decided to use calcium lactate tablets as the test tablet for further investigation employing surfactants.

Method of Incorporating Surfactant.—Several methods were evaluated, using Aerosol OT as the surfactant and calcium lactate as the test tablet, as follows: (*a*) surfactant dissolved in granulating liquid, (*b*) starch previously moistened with alcoholic solution of surfactant and then dried, and (*c*) alcoholic solution of surfactant sprayed by means of an atomizer onto granulation contained in a revolving tablet coating pan. Methods (*a*) and (*c*) were evaluated with no starch and with 10% starch present. Method (*c*) was found to be the best method as shown by the shortest disintegration time (Table III). Furthermore, method (*c*) is applicable to most tablet formulations, while methods (*a*) and (*b*) require a pregranulation moistening process. Various concentrations of the surfactant and starch were evaluated. It was found that 0.2% (of tablet weight) of surfactant and 10% (of tablet weight) of starch were the best concentrations as shown by the shortest disintegration time (Table IV). In method (*c*), it was found that the volume of solution to be sprayed should be approximately $^1/_3$ the volume of granulating liquid required for proper moistening. A greater volume resulted in excessive softening of the granulation. A maximum volume was desirable in order to obtain uniform and complete coating of the granules. The sprayed granulation was dried

* Received May 3, 1957, from the School of Pharmacy, University of North Carolina, Chapel Hill.

Presented to the Scientific Section, A. PH. A., New York meeting, April-May, 1957.

Abstracted in part from a dissertation submitted by Ben F. Cooper to the Graduate School of the University of North Carolina, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in pharmacy.

The authors wish to express appreciation to the following firms who gratuitously supplied the surfactants employed in this study: American Cyanamid Co., Atlas Powder Co., Dewey and Almy Co., E. F. Drew Co., E. I. du Pont de Nemours Co., The Emulsol Corp., Armour and Co., Antara Chemicals Division of General Dyestuff Corp., Refined Products Corp., Wyandotte Chemicals Corp., Alrose Chemical Co., Monsanto Chemical Co., Rohm and Haas Co., and Victor Chemical Works.

† Fellow of the American Foundation for Pharmaceutical Education. Present address: Assistant Professor of Pharmacy, School of Pharmacy, University of North Carolina.

‡ Dean and Professor of Pharmacy, School of Pharmacy, University of North Carolina.

TABLE I.—TABLET FORMULATIONS

Active Medicament	Method of Manufacture	Granulating Agent	Lubricant	Punch Size, (in.)	Weight (gr.)	Hardness (Kg.)
Acetylsalicylic Acid	Double compression	None	None	3/8	5	7.0
A. P. C.	Double compression	None	None	7/16	6 1/2	5.0
Aluminum Hydroxide	Wet granulation	20% Acacia mucilage	1% Magnesium stearate	3/8	7 1/2	6.0
Calcium Lactate	Wet granulation	Alcohol	1% Magnesium stearate	3/8	5	7.0
Sodium Salicylate	Wet granulation	20% Gelatin solution	2% Magnesium stearate	3/8	5	6.0
Sulfamerazine	Wet granulation	20% Acacia mucilage	3% Talc	3/8	5	7.0
Thyroid	Wet granulation	20% Acacia mucilage	3% Talc	9/32	1[a]	7.0
Ammonium Chloride	Direct compression	None	None	3/8	5	4.0
Sodium Bicarbonate	Wet granulation	20% Acacia mucilage-3% Acacia powder	1% Magnesium stearate	11/32	5	4.0
Sodium Chloride	Direct compression	None	None	3/8	7 1/2	6.0
Tricalcium Phosphate	Wet granulation	Water	1% Magnesium stearate	3/8	5	7.0
Magnesium Oxide	Wet granulation	Alcohol	1% Magnesium stearate	3/8	5	7.0
Magnesium Trisilicate	Wet granulation	15% Acacia mucilage	1% Magnesium stearate	13/32	5	7.0
Sulfathiazole	Wet granulation	10% Glucose solution	1% Magnesium stearate	3/8	5	6.0

[a] Plus 2 gr. lactose as diluent.

TABLE II.—PRELIMINARY TEST TABLETS

Active Medicament	Disintegrating Agent (%)	Disintegration Time (sec.)
Acetylsalicylic Acid	None	>3,600.0
Acetylsalicylic Acid	Starch 10	36.5
A. P. C.	None	>3,600.0
A. P. C.	Starch 10	9.5
Aluminum Hydroxide	None	>3,600.0
Aluminum Hydroxide	Starch 10	139.0
Calcium Lactate	None	1,192.0
Calcium Lactate	Starch 10	1,219.5
Sodium Salicylate	None	431.0
Sodium Salicylate	Starch 10	1,080.0
Sulfamerazine	None	>3,600.0
Sulfamerazine	Starch 10	158.5
Thyroid	None	895.5
Thyroid	Starch 10	229.5

TABLE III.—CALCIUM LACTATE TABLETS CONTAINING AEROSOL OT

Method of Incorporating Aerosol OT	Disintegration Time (sec.)
Aerosol OT in granulating liquid	876.0
Starch plus Aerosol OT in granulating liquid	645.0
Starch moistened with Aerosol OT solution	1,047.5
Aerosol OT solution applied by spraying	743.0
Starch plus Aerosol OT solution applied by spraying	486.0

at 48° for twenty-four hours and rescreened through a 16-mesh screen.

All subsequent formulations containing surfactants were prepared according to method (*c*) and contained 10% starch and 0.2% surfactant.

TABLE IV.—CALCIUM LACTATE TABLETS CONTAINING SELECTED DISINTEGRATING AGENTS

Disintegrating Agent, %	Disintegration Time (sec.)
10 Starch (control)	1,175.5
0.1 Aerosol OT	775.0
0.2 Aerosol OT	762.5
0.5 Aerosol OT	750.0
10 Starch, 0.1 Aerosol OT	595.0
10 Starch, 0.2 Aerosol OT	542.0
10 Starch, 0.5 Aerosol OT	575.5
5 Starch, 0.2 Aerosol OT	775.0
10 Starch,[a] 0.2 Aerosol OT	704.0

[a] Starch added after spraying procedure.

Evaluation of Selected Surfactants.—The surfactants employed in this study were selected to represent a wide range of products according to ionic classification, chemical composition, physical properties, uses, official status, and popularity. The results of incorporating 21 selected surfactants into calcium lactate tablets containing starch are tabulated in Table V.

The two most effective surfactants, Aerosol OT and Aerosol MA, were selected for further investigation. Table VI gives the results of evaluating various tablet formulations immediately after compression and after seven-month storage at room temperature in tight glass containers on an open shelf.

DISCUSSION

All tablets containing official drugs and 10% starch disintegrated within official time limits. However, the fact that a tablet meets official standards does not exclude the desirability for an improved

TABLE V.—PROPERTIES OF SELECTED SURFACTANTS

Surfactant	Disintegration Time of Calcium Lactate Tablets (sec.)	Physical Form	Solvent in Tablet Formulation	Ionic Classification	Surface Tension, 0.1% Solution at 25° (dynes/cm.) (uncorrected)
Aerosol OT	473.5	Wax	Alcohol	Anionic	30.4
Aerosol MA	498.0	Wax	Alcohol	Anionic	44.0
Aerosol OT-B	699.5	Powder	Alcohol	Anionic	31.2
Aerosol AY	749.0	Wax	Alcohol	Anionic	44.6
Detergent 1011	768.5	Liquid	Alcohol	Nonionic	28.6
Victorwet 12	771.0	Liquid	Alcohol	Nonionic	27.9
Tween 21	812.0	Liquid	Alcohol	Nonionic	34.2
Triton X-100	831.0	Liquid	Alcohol	Nonionic	31.5
Perma Par ACA-100	836.5	Paste	Alcohol	Cationic	26.4
Ethofat C/20	846.5	Liquid	Alcohol	Nonionic	33.6
Tween 81	878.5	Liquid	Alcohol	Nonionic	35.4
Santomerse D	895.5	Powder	Alcohol	Anionic	33.7
Igepal CTA-639	976.0	Liquid	Alcohol	Nonionic	30.8
Quaternary C	979.5	Paste	Alcohol	Cationic	33.8
Brij 35	984.5	Wax	Alcohol	Nonionic	43.5
Aerosol IB	986.5	Powder	Alcohol	Anionic	46.7
Daxad 11	995.5	Powder	Diluted alcohol	Anionic	70.5
Pluronic L64	1,025.0	Liquid	Alcohol	Nonionic	41.5
Duponol G	1,060.0	Paste	Alcohol	Anionic	30.4
Sodium Lauryl Sulfate	1,061.5	Powder	25% Alcohol	Anionic	30.3
Emcol MST	1,375.5	Wax	Ether	Nonionic	Insoluble

disintegration time. Furthermore, starch is not a satisfactory disintegrating agent for all tablets.

Incorporation of starch in sodium salicylate formulations hindered disintegration. Muller's theory (3) that starch forms a paste, enveloping soluble substances in such a way as to prevent ready solution of the tablet, could not be substantiated. A possible explanation may be suggested from gelatinization studies on starches (4). Several reagents in aqueous solutions cause starches to swell or gelatinize at ordinary temperatures. Sodium salicylate was found to be the most effective gelatinizing agent.

Improvement in tablet disintegration time was obtained by incorporating surfactants in certain tablet formulations (Table VI). Improvement was significantly greater in tablet formulations containing starch than in those containing no starch. A synergistic action appeared to exist between the surfactant and starch, resulting in a faster disintegration time when compared to tablets containing neither surfactant nor starch, surfactant alone, or starch alone. A possible explanation for this synergistic action may be suggested by the method of incorporating the surfactant and the starch. The best method of incorporating the surfactant consisted of spraying the granule surfaces with a volatile solution of the surfactant. The granules were thus coated with a film of surfactant. Assuming the identity of the granules was retained after compression (5, 6), the maximum amount of surfactant was continuously exposed to the disintegration medium at the granule interfaces. A reduction of surface tension at granule interfaces occurred, thereby promoting more rapid softening of the tablet, more rapid absorption of disintegration medium by starch, and subsequently more rapid disintegration of the tablet. In combination with surfactants, starch added before granulation gave better results than starch added after spraying. Starch added after spraying probably formed a coating on top of the surfactant coating, thus interfering with or delaying the action of the surfactant. In addition, the loose starch granules probably were not uniformly distributed throughout the tablet due to the tendency to classify or migrate downward during compression. The addition of a lubricant after spraying also probably delayed disintegration due to a protective coating formed around the granules. It would be desirable to use a surfactant having lubricating properties as well as the property of improving disintegration time. Of the surfactants studied which decreased disintegration time, none of them in the concentrations used was suitable as lubricants.

In general, no significant correlation between aging and change in disintegration time could be demonstrated. However, two specific tablets, magnesium oxide and tricalcium phosphate, showed a pronounced increase in disintegration time after aging (Table VI).

No significant correlation between disintegration time and surface tension values of aqueous solutions of the surfactants could be demonstrated. It is probable that any relationship between speed of disintegration and lowering of surface tension is qualitative rather than quantitative.

The four surfactants giving the shortest disintegration times (Aerosol OT, Aerosol MA, Aerosol OT-B®, and Aerosol AY®) are similar in certain chemical and physical properties (Table V). All are anionic in ionizing characteristics and all are waxes, except Aerosol OT-B. However, Aerosol OT-B contains 85% Aerosol OT (a waxy solid) and a waxy residue is formed when an alcoholic solution is evaporated to dryness. All four agents are closely related structurally as derivatives of sulfosuccinic acid.

SUMMARY

1. Satisfactory formulations are presented for tablets of acetylsalicylic acid, ammonium chlo-

TABLE VI.—SELECTED DISINTEGRATING AGENTS IN VARIOUS TABLET FORMULATIONS

Active Ingredient	% Disintegrating Agent	Hardness (Kg.) Original	Hardness (Kg.) 7-Month Storage	Disintegration Time (sec.) Original	Disintegration Time (sec.) 7-Month Storage
Calcium Lactate	None	6.8	6.9	1,192.0	1,328.0
	10 Starch	7.0	7.3	1,219.5	1,233.5
	0.2 Aerosol OT	6.2	7.3	743.0	799.5
	0.2 Aerosol MA	6.8	7.5	723.5	776.0
	10 Starch, 0.2% Aerosol OT	6.8	6.8	542.0	527.5
	10 Starch, 0.2% Aerosol MA	6.5	6.5	498.0	481.5
Sodium Salicylate	None	6.1	6.1	431.0	376.0
	10 Starch	6.5	6.9	1,080.0	1,086.5
	0.2 Aerosol OT	5.7	6.4	453.0	456.0
	0.2 Aerosol MA	5.7	6.1	505.5	507.0
	10 Starch, 0.2% Aerosol OT	6.0	6.5	1,074.0	945.5
	10 Starch, 0.2% Aerosol MA	6.6	7.4	1,196.5	1,060.5
Sodium Bicarbonate	None	3.9	6.4	1,149.5	1,637.5
	10 Starch	4.0	2.8	623.5	1,174.0
	0.2 Aerosol OT	3.9	6.4	675.0	765.5
	0.2 Aerosol MA	4.6	5.3	838.5	944.5
	10 Starch, 0.2% Aerosol OT	4.6	2.7	194.5	125.5
	10 Starch, 0.2% Aerosol MA	4.3	2.7	205.0	171.0
Tricalcium Phosphate	None	6.7	6.1	>3,600.0	>3,600.0
	10 Starch	7.5	7.3	>3,600.0	>3,600.0
	0.2 Aerosol OT	7.0	5.7	>3,600.0	>3,600.0
	0.2 Aerosol MA	7.2	6.0	>3,600.0	>3,600.0
	10 Starch, 0.2% Aerosol OT	7.6	7.2	15.5	>3,600.0
	10 Starch, 0.2% Aerosol MA	7.5	7.1	15.5	535.0
Magnesium Oxide	None	7.2	8.5	>3,600.0	>3,600.0
	10 Starch	7.2	7.3	990.0	>3,600.0
	0.2 Aerosol OT	6.8	9.2	>3,600.0	>3,600.0
	0.2 Aerosol MA	6.7	7.8	>3,600.0	>3,600.0
	10 Starch, 0.2% Aerosol OT	6.8	7.4	194.5	2,370.0
	10 Starch, 0.2% Aerosol MA	6.8	7.9	115.5	>3,600.0
Magnesium Trisilicate	None	6.7	6.4	3,292.5	>3,600.0
	10 Starch	6.9	6.0	515.0	400.0
	0.2 Aerosol OT	7.0	5.9	3,082.0	>3,600.0
	0.2 Aerosol MA	6.7	5.7	3,031.0	>3,600.0
	10 Starch, 0.2% Aerosol OT	7.4	6.4	323.5	338.0
	10 Starch, 0.2% Aerosol MA	6.5	5.6	231.0	281.0
Acetylsalicylic Acid	None	7.5	11.4	>3,600.0	>3,600.0
	10 Starch	7.5	11.9	36.5	36.0
	0.2 Aerosol OT	7.1	5.7	>3,600.0	>3,600.0
	0.2 Aerosol MA	7.5	5.8	>3,600.0	>3,600.0
	10 Starch, 0.2% Aerosol OT	7.6	8.7	19.0	32.0
	10 Starch, 0.2% Aerosol MA	7.1	7.8	13.5	15.5
A. P. C.	None	5.6	8.5	>3,600.0	>3,600.0
	10 Starch	5.4	7.4	9.5	19.0
	0.2 Aerosol OT	5.7	9.0	>3,600.0	>3,600.0
	0.2 Aerosol MA	5.6	10.1	>3,600.0	>3,600.0
	10 Starch, 0.2% Aerosol OT	4.7	7.2	11.5	39.0
	10 Starch, 0.2% Aerosol MA	4.8	7.6	10.5	20.0
Sulfathiazole	None	6.6	11.1	>3,600.0	>3,600.0
	10 Starch	6.0	6.5	21.0	29.0
	0.2 Aerosol OT	5.1	5.2	>3,600.0	>3,600.0
	0.2 Aerosol MA	5.1	5.9	>3,600.0	>3,600.0
	10 Starch, 0.2% Aerosol OT	5.8	5.7	45.5	73.0
	10 Starch, 0.2% Aerosol MA	5.8	5.9	33.5	43.0
Ammonium Chloride	None	4.5	6.3	67.0	65.5
	10 Starch	3.3	3.8	7.5	11.5
	0.2 Aerosol OT	4.4	14.3	135.0	141.5
	0.2 Aerosol MA	4.0	14.3	118.0	114.0
	10 Starch, 0.2% Aerosol OT	3.3	3.3	6.5	5.5
	10 Starch, 0.2% Aerosol MA	3.3	3.3	5.5	6.0
Sodium Chloride	None	6.2	8.8	203.5	228.5
	10 Starch	5.5	6.7	164.5	147.0
	0.2 Aerosol OT	5.5	11.1	233.0	249.0
	0.2 Aerosol MA	5.3	8.0	204.0	203.5
	10 Starch, 0.2 Aerosol OT	Too Soft for Use			
	10 Starch, 0.2% Aerosol MA	Too Soft for Use			

ride, A. P. C., aluminum hydroxide, calcium lactate, tricalcium phosphate, magnesium oxide, magnesium trisilicate, sodium bicarbonate, sodium chloride, sodium salicylate, sulfamerazine, and sulfathiazole.

2. It was found that a specific incompatibility existed between sodium salicylate and starch as shown by a decreased disintegration rate.

3. Surfactants were generally effective in improving tablet disintegration time when sprayed onto granulations containing starch. Ten per cent of starch in combination with 0.2% surfactant was most effective. Improvement was obtained for tablets of calcium lactate, sodium bicarbonate, tricalcium phosphate, magnesium oxide, magnesium trisilicate, acetylsalicylic acid, and ammonium chloride. No improvement was obtained for tablets of sodium salicylate, A. P. C., and sulfathiazole.

4. Evaluation results of 21 surfactants are presented. Aerosol OT and Aerosol MA were most effective in reducing tablet disintegration time. Aerosol MA was slightly superior to Aerosol OT.

5. The four most efficient surfactants which were evaluated as disintegrating agents are anionic in ionization, waxy solids, and structurally related to sulfosuccinic acid.

6. Upon storage, tablet hardness and disintegration rate changed unpredictably, but in most cases this change was not great.

REFERENCES

(1) Curlin, L. C., THIS JOURNAL, **44**, 16(1955).
(2) Wolff, J. E., DeKay, H. G., and Jenkins, G. L., *ibid.*, **36**, 407(1947).
(3) Muller, S. B., *Am. J. Pharm.*, **87**, 197 (1915).
(4) Anker, C. A., and Geddes, W. F., *Cereal Chem.*, **21**, 335 (1944).
(5) Chavkin, L., *Drug & Cosmetic Ind.*, **75**, 466(1954).
(6) Strickland, W. A., Jr., Nelson, E., Busse, L. W., and Higuchi, T., THIS JOURNAL, **45**, 51(1956).

An Investigation of the Incompatibilities of Quaternary Ammonium Germicides in Compressed Troches I.*

The Adsorption of Cetylpyridinium Chloride and Benzalkonium Chloride by Talc and Kaolin

By NICHOLAS H. BATUYIOS† and E. A. BRECHT

A method for determining the adsorptive capacity of talc and kaolin for cetylpyridinium chloride and benzalkonium chloride is described. The adsorption isotherms were determined at 25° ± 0.3° and the results plotted according to the Langmuir and Freundlich equations. The Langmuir constant *b* was calculated for talc-cetylpyridinium chloride and kaolin-cetylpyridinium chloride combinations. Talc and kaolin gave the same type of curves with both compounds. They differed only in adsorptive capacity. Cetylpyridinium chloride was adsorbed to a greater extent than benzalkonium chloride by kaolin and talc. The adsorption patterns of these two quaternaries were found to be different. Bacteriological and chemical tests indicated that the amount of cetylpyridinium chloride inactivated by 1 Gm. of talc is approximately 5 mg.

ALTHOUGH the antibacterial properties of the quaternary ammonium germicides were recognized as early as 1908 by Einhorn and Gottler (1, 2), it remained for Domagk (3) in 1935 to demonstrate and focus attention on the high antibacterial activity of benzalkonium chloride.

There are a number of qualities which make these compounds acceptable as disinfectants. They contain no phenols, iodine, active chlorine, mercury, or other heavy metals. The commonly used quaternary ammonium germicides are odorless. They are extremely stable chemically and bacteriologically (4). Solutions remain stable over long periods of storage, are nonvolatile and unaffected by freezing, boiling, autoclaving, and light. In general they are soluble in water and

* Received May 3, 1957, from the School of Pharmacy, University of North Carolina, Chapel Hill.
Presented to the Scientific Section, A. PH. A., New York meeting, April-May, 1957.
Abstracted in part from a dissertation by Nicholas H. Batuyios, submitted to the Graduate School of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
The authors are indebted to Dr. T. C. Grubb and assistants of the Vick Chemical Company for the bacteriological tests.
† Vick Research Fellow. Present address: U. S. P. Post-Doctoral Fellow, College of Pharmacy, University of Michigan, Ann Arbor.

polar organic solvents (5, 6). Toxicological studies indicate that the germicidal quaternary ammonium compounds are nontoxic in bactericidal concentrations even when injested over long periods of time (7–11). Their popularity is demonstrated by their wide use as disinfectants and by the fact that since their reintroduction by Domagk more than 800 publications have appeared in which mention has been made of the use of this class of disinfectants (12–14).

Germicidal quaternary ammonium compounds are effective against skin pathogens and oral bacteria in high dilutions (6). Their unique properties have stimulated much research to evaluate their usefulness in products designed for application to human skin and mucous membranes (6, 15, 16). They are used mainly in pharmaceutical preparations (17, 18) such as tinctures, solutions, ointments, creams, lotions, oils, powders, emulsions, tooth pastes and powders, and in hair rinses.

Commercial troches containing quaternary ammonium germicides are also available. The prime difficulty in this form of medication is the loss of activity due to the incompatibilities encountered in the formulation and compression. This investigation was undertaken with the purpose of formulating a troche by compression containing cetylpyridinium chloride in which the bactericidal activity will be maintained.

The quaternary ammonium germicides are inactivated when incorporated with certain substances. This inactivation is due mainly to the strong positive charge they bear when dissociated in solution. The inactivation may be evidenced by a physical change, but in some cases with no change in physical appearance complete loss of bactericidal activity occurs (3, 6, 12, 19–32).

The first step in this investigation was to determine the compatibility of cetylpyridinium chloride with the materials used in the compression of troches. Talc, which is a commonly used lubricant, inactivates these compounds. To determine the extent of inactivation, the adsorptive capacity of talc was determined by chemical and bacteriological means. Kaolin and benzalkonium chloride were included in the adsorption studies for comparative purposes.

EXPERIMENTAL

Materials.—Talc U. S. P. (Merck) was passed through a No. 270 sieve, dried at 110° for forty-eight hours and stored in an air-tight container. Kaolin N. F. (Colloidal, Merck) was dried at 110° for forty-eight hours and placed in an air-tight container. Kaolin could not be sieved because the particles agglomerated when shaken on the sieve. Cetylpyridinium chloride was obtained in crystalline form, whereas benzalkonium chloride was available in a 12.8% aqueous solution.

Adsorption.—In order to determine whether the amount of quaternary adsorbed is proportional to the weight of adsorbent, talc and kaolin were used in 1.500- and 3.000-Gm. quantities. Cetylpyridinium chloride and benzalkonium chloride were standardized according to the U. S. P. XV method for benzalkonium chloride and then diluted to the following concentrations at 25°:

1:3333 (15 mg./50 ml.)
1:2500 (20 mg./50 ml.)
1:2000 (25 mg./50 ml.)
1:1428 (35 mg./50 ml.)
1:1000 (50 mg./50 ml.)
1:500 (100 mg./50 ml.)
1:250 (200 mg./50 ml.)

The adsorbent was weighed and then placed in a 60-ml. centrifuge tube with a constricted neck having a length of 12 cm. and outside diameter of 3 cm. To this, 50.00 ml. of the quaternary solution was added at 25° and sealed by means of a rubber cap. Four tubes were secured vertically at one time to the mixing apparatus (33) and allowed to tumble at 25° ± 0.3° in a constant temperature bath until equilibrium was reached. One hour of mixing was adequate to reach equilibrium, but in all experiments the time of mixing was extended to three hours. The speed of the mixing apparatus was adjusted to about 80 r. p. m. At greater speeds the adsorbent settled at the bottom of the tubes due to centrifugal force. To insure uniform mixing three glass beads were placed in each tube which prevented the adsorbent from adhering to the walls of the tubes. After mixing, the material was centrifuged at 1,450 r. p. m. for one-half hour, and 25.00 ml. of the clear supernatant liquid at 25° was pipetted into a 250-ml. Erlenmeyer flask, and assayed for quaternary content according to the Boyd-Carkhuff method (34). All experiments were run in duplicate. The adsorption isotherms are presented in Tables I–IV, and Figs. 1–6.

In order to determine whether the cetylpyridinium chloride adsorbed by talc was held irreversibly, the effect of successive washings on the initial amount adsorbed was investigated. Solutions of cetylpyridinium chloride of 1:2000-, 1:1000-, and 1:500-strength were mixed with 3.000 Gm. of talc, each, for three hours in the constant temperature bath. Then, they were centrifuged and the supernatant liquids were decanted and assayed individually for quaternary content. To the residues, 50.00-ml. portions of distilled water were added, mixed in the constant temperature bath for one hour, centrifuged, decanted, and assayed. The same process was repeated 10 times with each solution. The results are presented in Fig. 1.

Surface Tension.—The surface tension determinations were performed at 25°. The solutions were allowed to stand for at least one week before the surface tension was determined. Some of the values were checked after a period of seven months and the difference was found to be within 1%. The results are shown in Table V.

Bacteriological Tests.—The test procedure is as follows: Five ml. of the material to be tested is

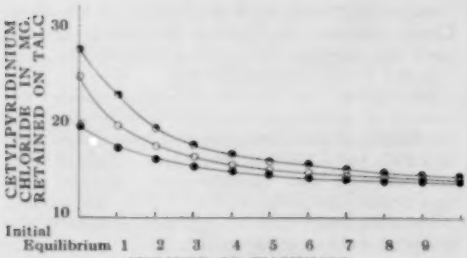


Fig. 1.—Cetylpyridinium chloride retained on 3.000 Gm. talc per 50.00 ml. at initial equilibrium and through repeated washings with 50.00-ml. portions of distilled water at 25°. ◓—100 mg. of cetylpyridinium chloride/50.00 ml., ⊙—50 mg. of cetylpyridinium chloride/50.00 ml., ●—25 mg. of cetylpyridinium chloride/50.00 ml.

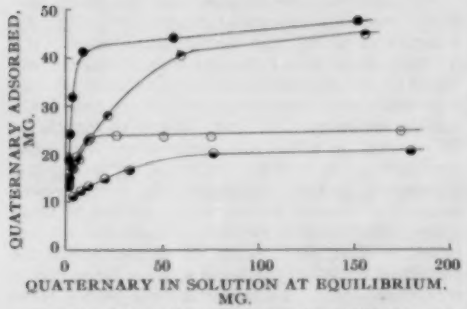


Fig. 2.—Adsorption isotherms for 3.000 Gm. adsorbent in 50.00 ml. at 25°. ●—Cetylpyridinium chloride and kaolin. ◓—Benzalkonium chloride and kaolin. ⊙—Cetylpyridinium chloride and talc. ◒ Benzalkonium chloride and talc.

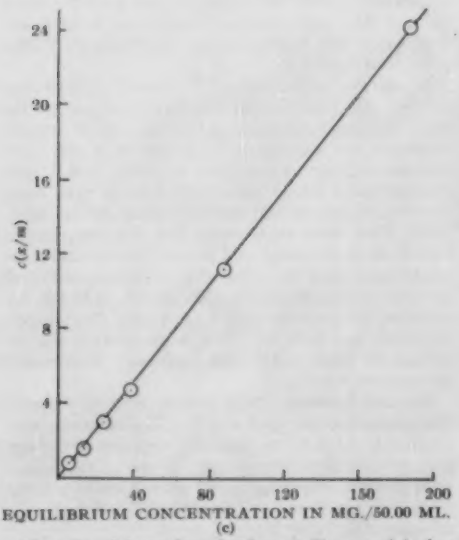


Fig. 3.—Adsorption isotherm (Langmuir) for 1.500 Gm. talc and cetylpyridinium chloride at 25°.

placed in a water bath at 37° for a short time to bring the solution to temperature. Then, 0.5 ml. of a twenty-four hour *Staphylococcus aureus* broth culture is added and a loopful of the mixture is transferred to leethen broth (to inactivate the quaternary) at intervals of three, five, and seven minutes after adding the test organism. The subculture tubes are incubated twenty-four hours at 37° and the presence or absence of the growth is recorded.

The samples were sent periodically to the testing laboratory in groups and each table, Tables VI–IX, represents the results obtained from a certain group. When the sample was a suspension, it was tested as such, unless otherwise indicated. The suspensions

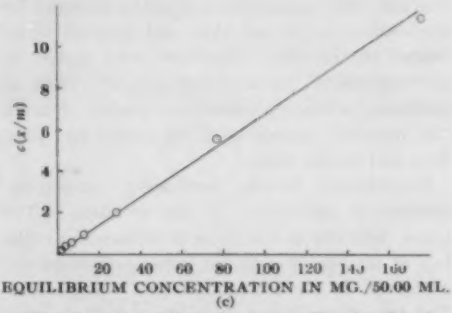


Fig. 4.—Adsorption isotherm (Langmuir) for 1.500 Gm. kaolin and cetylpyridinium chloride at 25°.

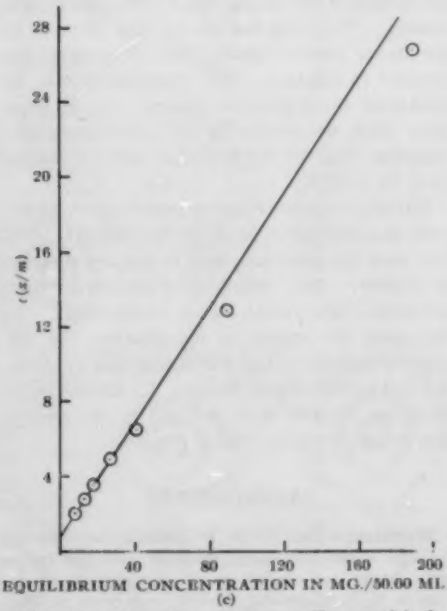


Fig. 5.—Adsorption isotherm (Langmuir) for 1.500 Gm. talc and benzalkonium chloride at 25°

were shaken for one-half hour on a mechanical shaker before being sent for testing.

DISCUSSION

The data, Tables I–IV and Fig. 2, show that the adsorption reaches a saturation point at high concentrations. The curves, Fig. 2, have the form which can be best represented by the Langmuir equation (35). This equation may be written as $x/m = abc/(1 + ac)$ or $c/(x/m) = (1/ab) + c/b$, where a and b are constants, x/m is the amount in mg. of the quaternary adsorbed per Gm. of adsorbent, and c is the equilibrium concentration of quaternary in mg. per 50.00 ml. $c/(x/m)$ may be plotted as a linear function of c with a slope of $1/b$ and having an intercept of $1/ab$. From this equation it is apparent that at high values of c, x/m approaches the constant b, and adsorption is approximately independent of the concentration. Therefore, the value of the constant b is an indication of the adsorptive capacity of the adsorbent.

The constant b of the Langmuir equation was calculated for combinations of talc or kaolin, and cetylpyridinium chloride from the linear curves, Figs. 3 and 4. These values are shown in Table X which includes also the values obtained using 3.000 Gm. talc and kaolin. The adsorption isotherms, Figs. 5 and 6, of talc or kaolin, and benzalkonium chloride did not follow the Langmuir equation in all concentrations studied, therefore, no attempt was made to calculate the constant b.

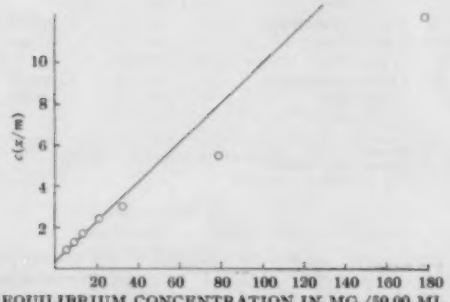


Fig. 6.—Adsorption isotherm (Langmuir) for 1.500 Gm. kaolin and benzalkonium chloride at 25°.

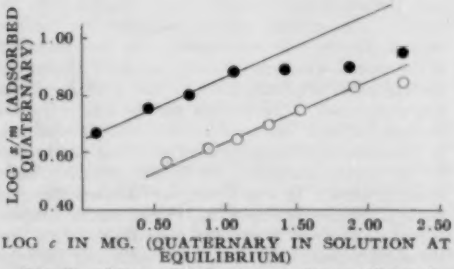


Fig. 7.—Adsorption isotherms (Freundlich) for 3.000 Gm. talc at 25°. ●—Cetylpyridinium chloride. ⊙—Benzalkonium chloride.

The results of Table X indicate that the adsorptive capacity of kaolin for cetylpyridinium chloride is approximately twice that of talc. The experimental data also indicate that cetylpyridinium chloride is adsorbed to a greater extent than benzalkonium chloride by both kaolin and talc. This could possibly be due to difference in molecular orientation. Talc and kaolin give the same type of curves with either benzalkonium chloride or cetylpyridinium chloride. They differ only in adsorptive capacity.

The adsorption patterns of cetylpyridinium chloride and benzalkonium chloride are different. Cetylpyridinium chloride exhibits a Langmuir type adsorption indicated by the linearity of the curves. Benzalkonium chloride does not follow the Langmuir equation at high concentrations. Figures 5 and 6 show that only concentrations weaker than 1:1,000 result in a straight line. Examination of the Langmuir adsorption curves for benzalkonium chloride, Figs. 5 and 6, and of the surface tension determinations of the same compound, Table V, revealed that the concentration range of 1:1,000 to 1:2,000, at which the surface tension began to increase, is the concentration range on the graphs at which the curves deviated from the straight line. The reason for these two anomalies may be the same and it is possibly related to the formation and the concentration of micelles.

With regard to surface tension Adam and Shute (36) observed the same anomaly with cetyltrimethylammonium bromide. Solutions less than 1:2,000

TABLE I.—ADSORPTION OF CETYLPYRIDINIUM CHLORIDE BY TALC AT 25°

Concn., mg./ 50.00 ml.	Talc, 3.000 Gm./ 50.00 ml., Amount Adsorbed, mg.	Talc, 1.500 Gm./ 50.00 ml., Amount Adsorbed, mg.
15	14.11	9.50
20	17.10	...
25	19.50	11.69
35	23.37	11.79
50	23.50	11.85
100	23.90	11.99
200	24.66	12.15

TABLE II.—ADSORPTION OF CETYLPYRIDINIUM CHLORIDE BY KAOLIN AT 25°

Concn., mg./ 50.00 ml.	Kaolin, 3.000 Gm./ 50.00 ml., Amount Adsorbed, mg.	Kaolin, 1.500 Gm./ 50.00 ml., Amount Adsorbed, mg.
15	14.89	13.17
20	19.65	15.77
25	24.17	18.09
35	31.71	21.55
50	41.66	21.52
100	44.00	21.10
200	46.74	23.28

TABLE III.—ADSORPTION OF BENZALKONIUM CHLORIDE BY TALC AT 25°

Concn., mg./ 50.00 ml.	Talc, 3.000 Gm./ 50.00 ml., Amount Adsorbed, mg.	Talc, 1.500 Gm./ 50.00 ml., Amount Adsorbed, mg.
15	11.10	6.30
20	12.50	6.90
25	13.20	7.40
35	14.70	8.20
50	16.80	9.50
100	20.50	10.50
200	20.90	10.60

TABLE IV.—ADSORPTION OF BENZALKONIUM CHLORIDE BY KAOLIN AT 25°

Concn., mg./ 50.00 ml.	Kaolin, 3.000 Gm./ 50.00 ml., Amount Adsorbed, mg.	Kaolin, 1.500 Gm./ 50.00 ml., Amount Adsorbed, mg.
15	13.84	9.43
20	16.81	10.58
25	19.36	11.67
35	22.85	13.55
50	27.88	16.78
100	40.44	21.56
200	43.09	21.84

TABLE V.—SURFACE TENSION DETERMINATIONS AT 25°

	Concn.	Dynes/ cm.
1. Cetylpyridinium Chloride	1:10,000	54.8
	1:5,000	46.5
	1:3,333	43.2
	1:2,500	43.9
	1:2,000	43.2
	1.1,000	43.3
	1:500	42.7
	1:250	43.4
	1.244%	42.2
	4.837%	39.4
2. Benzalkonium chloride	1:10,000	53.5
	1:5,000	39.0
	1:3,333	33.5
	1:2,500	32.8
	1:2,000	32.0
	1:1,000	33.0
	1:500	36.1
	1:250	36.5
	1.24%	37.0
	12.8%	36.7

attained a final surface tension of about 25 dynes per cm., whereas stronger solutions never went lower than 30 to 31 dynes per cm. No explanation was offered for this difference.

The adsorption results were also plotted according to Freundlich equation (35). As shown in Fig. 7, neither cetylpyridinium chloride nor benzalkonium chloride conform to the Freundlich adsorption isotherm over the whole concentration range studied. This is indicated by the failure to obtain straight lines when the logs of the amounts adsorbed (x/m) were plotted against the equilibrium concentrations (c). The curves obtained with cetylpyridinium chloride and kaolin and benzalkonium chloride and kaolin are of the same type except that the breaks in the curves occurred at lower equilibrium concentrations.

TABLE VI.—BACTERIOLOGICAL EVALUATIONS

	Sample	Time Interval, min. 3	10	15	Comments
	Phenol[c] 1:80	+[b]	+	−[a]	Control
	1:90	+	+	+	
		3	5	7	
	Laboratory's 1:2,500 cetylpyridinium chloride[c]	+	−	−	Control
		+	−	−	
		+	−	−	
1	Benzalkonium chloride, 1:250	−	−	−	Control series
		−	−	−	
		−	−	−	
2	Benzalkonium chloride, 1:500	−	−	−	Control series
		−	−	−	
		−	−	−	
3	Benzalkonium chloride, 1:1,000	−	−	−	Control series
		−	−	−	
		−	−	−	
4	Benzalkonium chloride, 1:2,000	−	−	−	Control series
		−	−	−	
		−	−	−	
5	Benzalkonium chloride, 1:2,500	−	−	−	Control series
		−	−	−	
		−	−	−	
6	Benzalkonium chloride, 1:3,333	−	−	−	Control series
		−	−	−	
		−	−	−	
7	Cetylpyridinium chloride, 1:250	−	−	−	Control series
		−	−	−	
		−	−	−	
8	Cetylpyridinium chloride, 1:5,000	−	−	−	Control series
		−	−	−	
		−	−	−	
9	Cetylpyridinium chloride, 1:1,000	−	−	−	Control series
		−	−	−	
		−	−	−	
10	Cetylpyridinium chloride, 1:2,500	−	−	−	Control series
		−	−	−	
		−	−	−	

[a] Growth.
[b] Absence of growth.
[c] Vick Chemical Company.

Rose, *et al.* (37), studied the adsorption of certain cationic, anionic, and nonionic agents by carbon black. The adsorption of the compounds tested did not conform to the Freundlich adsorption isotherm over the whole concentration range studied (0.01%–0.2%). The critical micelle concentration for the anionic agents was determined. In the majority of the cases it was found that the breaks in the curves corresponded approximately to the critical micelle concentrations. The critical micelle concentration for the cationic compounds was not determined. It was concluded only by inference that the breaks in the curves corresponded to the critical micelle concentration. It was also observed that the portions of the curves above the critical micelle concentration appeared linear, in conformity with the Freundlich equation. According to Weiser (35) the empirical Freundlich equation is useless for the portion of the curve that runs parallel to the concentration axis. The results obtained in this investiga-

TABLE VII.—BACTERIOLOGICAL EVALUATIONS

		Time Interval, min. 1	3	5	7	Comments
	Phenol 1:80		+	+	+	Control
	1:90		+	+	+	
			3	5	7	
	Laboratory's 1:2,500 cetylpyridinium chloride		+	+	−	Control
			−	+	+	
			+	−	−	
		1	3	5	7	
11	Cetylpyridinium chloride, 1:1,000	+	−	+	−	Control
		+	−	−	−	
		+	+	−	−	
12	Cetylpyridinium chloride, 1:1,500	+	−	−	−	Control
		−	−	−	−	
		+	+	−	−	
13	Cetylpyridinium chloride, 1:2,000	+	−	−	−	Control
		−	−	−	−	
		+	−	−	−	
14	Cetylpyridinium chloride, 1:3,000	+	−	−	−	Control
		+	−	−	−	
		+	+	−	−	
15	Cetylpyridinium chloride, 1:4,000	+	+	+	+	Control
		+	+	−	−	
		+	+	+	−	
16	Total volume of 50 ml. containing 4.00 Gm. talc and 50 mg. cetylpyridinium chloride (1:1,000)	−	−	−	−	No apparent inactivation by talc
		+	+	−	+	
		−	−	−	−	
17	Total volume of 50 ml. containing 10.00 Gm. talc and 75 mg. cetylpyridinium chloride (1:667)	+	−	−	−	No apparent inactivation by talc
		+	−	−	−	
		+	+	+	−	

TABLE VIII.—BACTERIOLOGICAL EVALUATIONS

		Suspension			Supernatant Liquid			
		Time Interval, min. 3	5	7	3	5	7	Comments
18	Cetylpyridinium chloride, 1:1,000	..	..	..	−	−	−	Control
		..	..	..	+	−	+	
		..	..	..	−	−	−	
19	Total volume of 50 ml. containing 2.50 Gm. kaolin and 50 mg. cetylpyridinium chloride (1:1,000)	+	−	−	−	+	−	No apparent inactivation by kaolin
		−	−	−	−	−	−	
		−	−	−	−	−	−	
20	Total volume of 50 ml. containing 2.50 Gm. kaolin residue which still contained approximately 20 mg. cetylpyridinium chloride after repeated washings with distilled water (1:2,500)	+	+	+	+	+	+	No release of activity from kaolin
		+	+	+	+	+	+	
		+	+	+	+	+	+	
21	Total volume of 50 ml. containing 6.00 Gm. talc and 50 mg. cetylpyridinium chloride (1:1,000)	−	−	−	−	−	−	The supernatant liquid, partly inactivated
		−	−	−	+	−	−	
		−	−	−	+	+	+	
22	Total volume of 50 ml. containing 2.50 Gm. talc and 50 mg. cetylpyridinium chloride (1:1,000)	−	−	−	−	−	−	No apparent inactivation by talc
		−	−	−	−	−	−	
		−	−	−	−	−	−	

tion, (Figs. 2 and 7) seem to be in complete agreement with Weiser's viewpoint.

Figure 1 indicates that a large portion of the amount of cetylpyridinium chloride held by talc at the initial equilibrium is released upon successive washings. Regardless of the original concentration of the quaternary, the amount retained on talc after the elutions seems to be the same. The fact that part of the amount originally adsorbed is released, indicates that probably the adsorption is physicochemical. The evidence presented here indicates that the amount of cetylpyridinium chloride retained irreversibly by 1 Gm. talc is about 5 mg.

The bacteriological tests presented in Tables VI–IX must be evaluated as a whole. It must be pointed out that the interpretation of the antibacterial activity from this type of test is difficult. Many variables (38) influence the results of these tests and conclusions can be drawn only after a series of tests have been made.

Since the quaternary ammonium germicides have surface-active properties, so-called "wild plusses" are always found in this type of test and one can

TABLE IX.—BACTERIOLOGICAL EVALUATIONS

	Sample	Time Interval, min. 3	10	15	Comments
	Phenol 1:80	+	−	−	Control
		+	+	−	
		3	5	7	
	Laboratory's 1:2,500 cetylpyridinium chloride	−	+	−	Control
		−	+	−	
		+	−	−	
23	Total volume of 50 ml. containing 0.80 Gm. talc and 20 mg. cetylpyridinium chloride (1:2,500)	+	+	−	Slight apparent inactivation by talc
		+	+	−	
		−	−	−	
24	As sample 23, except that it contains 1.60 Gm. talc (1:2,500)	+	−	−	Slight apparent inactivation by talc
		+	+	+	
		−	−	−	
25	As sample 23, except that it contains 2.00 Gm. talc (1:2,500)	−	−	−	Slight apparent inactivation by talc
		+	−	−	
		+	+	−	
26	As sample 23, except that it contains 4.00 Gm. talc (1:2,500)	+	+	+	Complete inactivation by talc
		+	+	+	
		+	+	+	
27	Cetylpyridinium chloride, 1:2,500	−	−	−	Control
		−	−	−	
		−	−	−	
28	Total volume of 50 ml. containing 10.00 Gm. talc and 50 mg. cetylpyridinium chloride (1:1,000)	+	+	+	Complete inactivation by talc
		+	+	+	
		+	+	+	
29	Cetylpyridinium chloride, 1:4,000	+	+	+	Control
		+	−	+	
		+	−	−	

only judge the results from the overall picture. The time interval at which the plus is found helps to determine whether the plus is wild. The tests in Table VI were run with the purpose of comparing cetylpyridinium chloride and benzalkonium chloride bacteriologically and of relating the surface tension to the bactericidal activity of both compounds. It can be said that no apparent relationship exists between surface tension and bactericidal activity.

The data in Table VIII indicate that the kaolin residue which is known to contain 20 mg. of the germicide in 50 ml. of solution failed to show antibacterial activity. This solution would be 1:2,500, if the cetylpyridinium chloride were released, and it would definitely show bactericidal activity. This indicates that the 20 mg. is held irreversibly by kaolin. The behavior of sample 21 indicates that part of the cetylpyridinium chloride adsorbed is released. This is shown very clearly by the fact that the suspension is more active than the supernatant liquid. By comparing samples 15, 16, and 17 it is evident that samples 16 and 17 show greater antibacterial activity than sample 15. Therefore, even though the talc inactivates part of the quaternary, the amount left gives a more active solution than the sample 15. Sample 15 contains 12.5 mg./50 ml. The samples 16 and 17 must contain more than 12.5 mg. after partial inactivation by talc, because their antibacterial activity is greater. If it is accepted that 1 Gm. of talc will inactivate about 5 mg. of cetylpyridinium chloride, as shown by the chemical determinations, then samples 16 and 17, after inactivation, should still contain 30 and 25 mg. of cetylpyridinium chloride, respectively. The samples 16 and 17 seem to behave similarly to all samples in Table VII, with the exception of sample 15. Samples 26 and 28 show complete inactivation. Samples 23, 24, and 25 show partial inactivation. The chemical and bacteriological determinations indicate that 1 Gm. of talc will inactivate approximately 5 mg. of cetylpyridinium chloride.

TABLE X.—CONSTANT *b* OF THE LANGMUIR EQUATION FOR CETYLPYRIDINIUM CHLORIDE

Adsorbent	Quantity, Gm.	Constant *b*
Talc	1.50	8.0
	3.00	8.2
Kaolin	1.50	15.4
	3.00	15.4

SUMMARY

1. A suitable method is described for the evaluation of the adsorptive capacity of talc and kaolin for cetylpyridinium chloride and benzalkonium chloride.

2. Talc and kaolin gave the same type of curves with either benzalkonium chloride or cetylpyridinium chloride. They differ only in adsorptive capacity.

3. The Langmuir constant *b* was found to be 8.1 and 15.4 for talc and kaolin with cetylpyridinium chloride, respectively.

4. Cetylpyridinium chloride was adsorbed by talc and kaolin to greater extent than benzalkonium chloride.

5. Cetylpyridinium chloride exhibited a Langmuir type adsorption. Benzalkonium chloride

did not follow the Langmuir equation at concentrations greater than 1:1,000. The adsorption of neither compound conformed to the Freundlich adsorption isotherm at high concentrations.

6. Benzalkonium chloride solutions showed an anomaly with respect to surface tension. This anomaly seemed to be related to the failure of the application of the Langmuir equation to the adsorption determinations of benzalkonium chloride.

7. Chemical and bacteriological tests indicated that 1 Gm. of talc inactivated approximately 5 mg. of cetylpyridinium chloride by irreversible adsorption.

REFERENCES

(1) Einhorn, A., and Gottler, M., *Ann. Chem.*, **361**, 150(1908).
(2) Jacobs, W. A., and Heidelberger, M., *J. Biol. Chem.*, **21**, 403(1915).
(3) Domagk, G., *Deut. med. Wochschr.*, **61**, 829(1935).
(4) Mueller, W. S., Bennett, E., and Fuller, J. E., *J. Dairy Sci.*, **29**, 751(1946).
(5) Lesser, M. A., *Drug & Cosmetic Ind.*, **64**, 434 and 559 (1949).
(6) Botwright, W. E., *Am. Perfumer Essent. Oil Rev.*, **61**, 361(1953).
(7) Finnegan, J. K., and Dienna, J. A., *Soap Sanit. Chemicals*, **30**, 147(1954).
(8) Warren, M. R., Becker, T. J., Marsh, D. G., and Shelton, R. S., *J. Pharmacol. Exptl. Therap.*, **74**, 401(1942).
(9) Werner, H. W., and Thompson, C. R., *Federation Proc.*, **8**, 473(1949).
(10) Alfredson, B. V., Stiefel, J. R., Thorp, F., Jr., Baten, W. D., and Gray, M. L., THIS JOURNAL, **40**, 263(1951).
(11) Shelanski, H. A., *Soap Sanit. Chemicals*, **25**, 125(1949).
(12) Lawrence, C. A., *Soap, Perfumery & Cosmetics*, **27**, 370(1954).
(13) Lawrence, C. A., "Surface Active Quaternary Ammonium Germicides," Academic Press, Inc., New York, 1950, pp. 1, 40, and 145.
(14) Ressugan, J. C. L., "Quaternary Ammonium Compounds in Chemical Sterilization," United Trade Press, Ltd., 24 Bride Lane, London, E. C. 4, 1951, pp. 14–24, 30–36, and 72.
(15) Hart, F., and Huyck, C. L., THIS JOURNAL, **37**, 272 (1948).
(16) Prusak, L. P., and Mattocks, A. M., *ibid.*, **38**, 67 (1949).
(17) Doubleday, C., *Soap, Perfumery & Cosmetics*, **26**, 263, 455(1953).
(18) Lewis, W. F., *ibid.*, **28**, 642(1955).
(19) Zussman, H. W., *Am. Perfumer Essent. Oil Rev.*, **47**, 54(1945).
(20) Tice, L. F., and Pressman, R., THIS JOURNAL, **34**, 201(1945).
(21) Miller, O. H., *ibid. Pract. Pharm. Ed.*, **13**, 657(1952).
(22) Nixon, W., and Cheetham, M. W., *Pharm. J.*, **165**, 46(1950).
(23) Joslyn, D. A., Yaw, K., and Rawlins, A. L., THIS JOURNAL, **32**, 49(1943).
(24) Barker, H. E. R., *Australasian J. Pharm.*, **29**, 801 (1948).
(25) Putnam, F. W., and Neurath, H., *J. Am. Chem. Soc.*, **66**, 692(1944).
(26) Glassman, H., *Ann. N. Y. Acad. Sci.*, **53**, 91(1950–51).
(27) Matalon, R., Salton, M. R. J., and Cohen, M., *Nature*, **167**, 241(1941).
(28) Swiss Patent, 276557; through *Chem. Abstr.*, **47**, 1341 (1953).
(29) Valko, E. I., and Dubois, A. S., *J. Bacteriol.*, **47**, 15(1944).
(30) Lawrence, C. A., THIS JOURNAL, **37**, 57(1948).
(31) Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exptl. Med.*, **74**, 621(1941).
(32) Quisno, R., Gibby, I. W., and Foter, M. J., *Am. J. Pharm.*, **118**, 320(1946).
(33) Hawkins, D., and Thompson, H. O., THIS JOURNAL, **42**, 424(1953).
(34) Carkhuff, E. D., and Boyd, W., *ibid.*, **43**, 240(1954).
(35) Weiser, H. B., "Colloid Chemistry," 2nd ed., John Wiley and Sons, New York, 1949, pp. 49, 68–88.
(36) Adam, N. K., and Shute, H. L., *Trans. Faraday Soc.*, **34**, 758(1938).
(37) Rose, G. R. F., Weatherburn, A. S., and Bayley, C. H., *Textile Research J.*, **21**, 427(1951).
(38) Davies, G. E., *Pharm. J.*, **170**, 277(1953).

Stability of Atropine in Aqueous Solution*

By ALBERT A. KONDRITZER and PETER ZVIRBLIS

A kinetic study of the proton catalyzed hydrolysis of atropine has been carried out and the temperature dependency of this reaction established. The constants obtained for this reaction have been combined with those previously reported for the hydroxyl catalyzed reactions and an equation proposed to predict the half life of atropine undergoing hydrolysis at constant pH and temperature. The pH of minimum hydrolysis has been calculated and found to vary with temperature from pH 4.11 at 0° to pH 3.24 at 100°. Calculated half lives of atropine in solution over the region of pH 2 to 7 at temperatures from 20° to 100° are given.

WE ARE engaged in a program to identify and evaluate the factors involved in the deterioration of aqueous solutions of atropine and its salts. A previous study (1) of the chemical kinetics of the alkaline hydrolysis of atropine in aqueous solution has shown that the mechanism of this hydrolysis consists of two reactions: one involving the unprotonated (base) form, and the other the protonated (ionic) form of the alkaloid. The effect of each reaction is governed by the hydroxyl-ion concentration and the temperature.

We have now studied the kinetics of the hydrolytic reaction catalyzed by hydrogen ions and correlated the resultant data with that obtained from the hydroxyl-ion catalysis. The pH for maximum stability to hydrolysis of sterile aqueous solutions of atropine and its salts has been calculated, as well as the influence of temperature.

HISTORICAL

The hydrolysis of atropine on heating either with HCl or $Ba(OH)_2$ solution was demonstrated by

* Received February 25, 1957, from the Physiology Division, Directorate of Medical Research, Army Chemical Center, Md.
Presented to the Scientific Section, A. PH. A., New York meeting, April–May, 1957.

Kraut in 1864 (2) and others (3). Since then the deterioration of atropine in aqueous solution and the greater influence of alkalinity compared to acidity on this process seem to be generally recognized, although until recently (1) quantitative data have been lacking and there has been no agreement concerning the precise range of pH favorable to maximum stability. Stability and resistance to acid and alkali, up to pH 9.2, has been claimed for solutions of atropine salts as well as to heating at 100° for thirty minutes (4). Atropine nitrate and phosphate were found to be more stable in aqueous solutions than atropine sulfate (5). A 20% hydrolysis of atropine at pH 5 at 80° in five hours has been reported (6); on the other hand no appreciable decomposition was found on sterilization for twenty minutes at 120° until pH 6, or over, was reached, when hydrolysis became very rapid (7). The preservation of aqueous atropine solutions by buffering at pH 4–5 has been recommended (8). Stability is claimed for 0.1% sterilized atropine sulfate solution in storage if the pH is kept in the range 2.8–6.0 (9), distinct deterioration on storage occurring if the pH is kept at 7 or higher. An isotonic atropine-borax-boric acid solution deteriorated 44% in one month (10). A 0.1% solution of atropine sulfate in water was found to be stable for at least four months when stored in resistance glass, whereas in lime glass 90% deterioration occurred in three weeks (11). In addition to deterioration caused by chemical and physical factors instability of atropine in solution has been attributed to the presence of fungus contamination as the result of the complete loss of pharmacological activity in a three-day old atropine solution (12).

THEORETICAL

If (AtH^+) represents the concentration of the protonated form of atropine and k_{H^+} the catalytic constant of the hydrogen ion catalysis (13), then

$$-\frac{d\,(AtH^+)}{dt} = k_{H^+}(H^+)(AtH^+) \quad \text{(Eq. 1)}$$

or,

$$-\frac{d\ln(AtH^+)}{dt} = k_{H^+}\,(H^+) \quad \text{(Eq. 2)}$$

Since the right side of Eq. 2 is a constant at constant pH, the rate of hydrolysis will be kinetically of the first order with respect to drug concentration, with a velocity constant

$$k = k_{H^+}\,(H^+) \quad \text{(Eq. 3)}$$

The most general expression for the observed velocity constant of reactions catalyzed by both hydrogen and hydroxyl ions (13) is

$$k = k_0 + k_{H^+}\,(H^+) + k_{OH^-}(OH^-) \quad \text{(Eq. 4)}$$

where k_0 represents the velocity constant of the "spontaneous" reaction, i. e., catalysis by the solvent molecules, and for water solutions equals $k_{H_2O}(H_2O)$. Hydrolytic reactions catalyzed by both hydrogen and hydroxyl ions, with no detectable water reaction, include hydrolysis of carboxylic esters (13–15). The results reported by Bullock and Carnell (16) indicate this to be true for the hydrolysis of procaine. Assuming that the hydrolytic reactions of atropine are included in this category and introducing the ionic product of water, $k_w = (OH^-)(H^+)$, Eq. 4, can be written

$$k + k_{H^+}(H^+) + k_{OH^-}\frac{K_w}{(H^+)} \quad \text{(Eq. 5)}$$

Between the regions of high and low pH the velocity will pass through a minimum at a hydrogen ion concentration which is found by differentiating k with respect to (H^+) in Eq. 5 and equating to zero (17), thus

$$k_{H^+} - K_W\frac{k_{OH^-}}{(H^+)^2} = 0, \quad \text{or} \quad (H^+)^2_M = K_w\frac{k_{OH^-}}{k_{H^+}} \quad \text{(Eq. 6)}$$

where $(H^+)_M$ is the hydrogen ion concentration at minimum velocity.

The value of k_M, the velocity constant at $(H^+)_M$, is obtained by substituting in Eq. 5 the value of $k_{OH^-}K_w$ from Eq. 6.

$$k_M = 2\,k_{H^+}(H^+)_M \quad \text{(Eq. 7)}$$

EXPERIMENTAL

Scope of the Studies.—The hydrolysis of atropine in perchloric acid solutions varying from 0.1 *N* to 0.4 *N* and at temperatures ranging from 70° to 90° was studied. The catalytic constant for the hydrogen ion catalyzed reaction was determined at the various temperatures and used to calculate the activation energy. With these values an equation was derived for predicting the rate of proton hydrolysis at any particular temperature and hydrogen ion concentration. By taking into account the effect of both hydroxyl and hydrogen ions the pH of minimum hydrolysis at various temperatures and the hydrolytic rates at these minimum pH values have been calculated. Also, the half lives of atropine solutions have been calculated for various pH values and temperatures.

Procedure.—A standardized solution of perchloric acid of the desired normality and containing 1 mg./ml. of atropine sulfate (U. S. P., twice recrystallized from ethanol; m. p. 189°–190° uncorr.) was kept at constant temperature in a thermostatically controlled bath. The hydrolytic reaction was followed by periodically removing 10-ml. samples for residual ester determination. The sample was made alkaline with ammonium hydroxide and the atropine base extracted with two 10-ml. portions of chloroform. The chloroform extracts were combined and extracted with exactly 10 ml. of 0.1 *N* HCl. The concentration of atropine in the acid solution was determined by ultraviolet absorption measurements at wavelengths 256.5 and 262 mμ using a Beckman model DU quartz spectrophotometer as previously described (1).

RESULTS

Influence of Ester Concentrations.—Velocity constants for atropine hydrolysis were determined in 0.099 *N*, 0.199 *N*, 0.301 *N*, and 0.401 *N* $HClO_4$ at 70.0°, 80.0° and 90.3°. Figures 1, 2, and 3 show that at constant temperature and hydrogen ion concentration the observed rate of hydrolysis of atropine

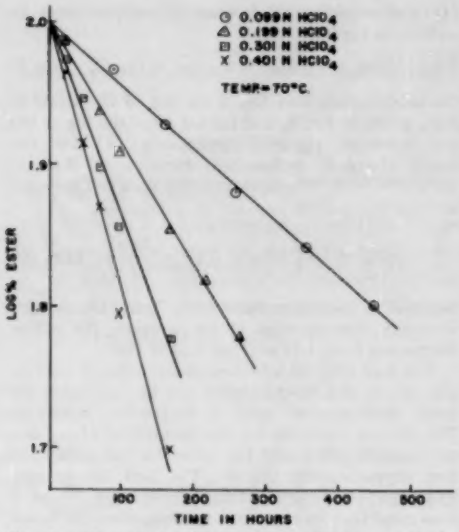


Fig. 1.—Hydrolysis of atropine in dilute perchloric acid solutions at 70°.

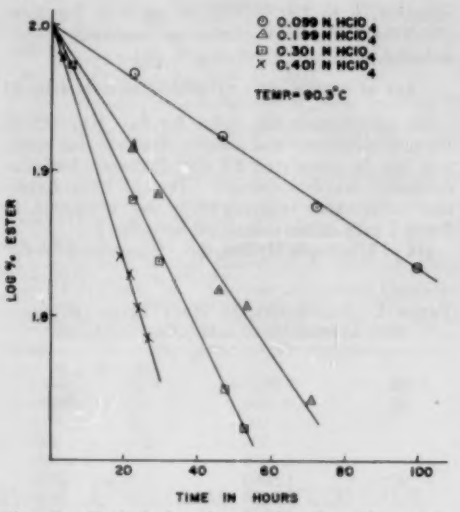


Fig. 3.—Hydrolysis of atropine in dilute perchloric acid solutions at 90.3°.

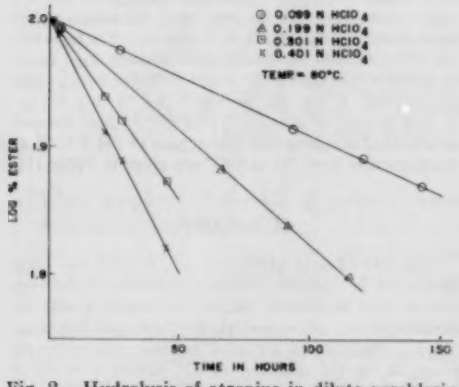


Fig. 2.—Hydrolysis of atropine in dilute perchloric acid solutions at 80°.

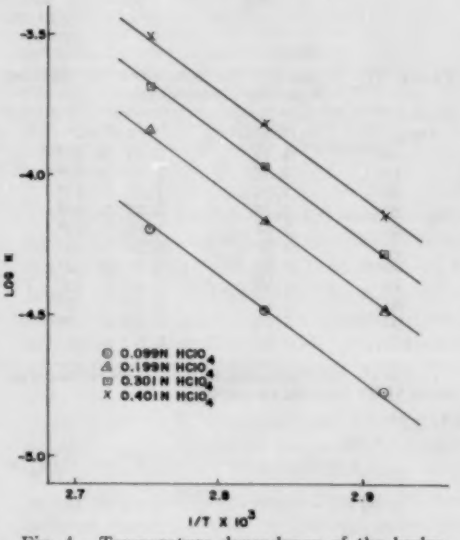


Fig. 4.—Temperature dependency of the hydrolytic reaction of atropine catalyzed by hydrogen ions. Log K min.$^{-1}$ *vs.* reciprocal of the absolute temperature.

is first order with respect to ester concentration. These results are in accord with Eq. 3.

Influence of Hydrogen Ion Concentration and Temperature Dependency.—The regression line for the velocity constants obtained at the various normalities was calculated for each temperature (18). A straight line relationship was obtained, showing the dependency of the rate of hydrolysis on the hydrogen ion concentration. The slope of the line for each temperature is equal to the respective catalytic constant. The values obtained for this constant are: k_{H^+} @ 70° = 1.754×10^{-4} min.$^{-1}$, k_{H^+} @ 80° = 3.668×10^{-4} min.$^{-1}$, and k_{H^+} @ 90.3° = 7.350×10^{-4} min.$^{-1}$

In Fig. 4 the velocity constants are plotted against the reciprocal of the respective absolute temperatures. The straight line relationships indicate that the mechanism responsible for the ester hydrolysis in acid solution is not altered with change in temperature. By subjecting the velocity constants to an analysis of covariance (18) the slope of the lines was found. The slope was set equal to $-Ea/2.303R$ and the energy of activation, Ea, was calculated to be 17,200 ± 500 calories per mole. By substituting both this value and the k_{H^+} at 70° in the Arrhenius

equation, $k = Ze^{-Ea/RT}$; or $\log k = \log Z - (Ea/2.303\ RT)$, Z, the Arrhenius frequency factor, is found to be 1.54×10^7 min.$^{-1}$, and

$$k_{H^+} = 1.54 \times 10^7\ e^{-17,200/RT}\ min.^{-1} \quad \text{(Eq. 8)}$$

By substituting this value for k_{H^+} into Eq. 3, the rate of hydrolysis of atropine due to proton catalysis can be calculated for any hydrogen ion concentration and temperature. The half lives of atropine determined experimentally are compared in Table I with values calculated from Eq. 3.

pH of Minimum Hydrolysis.—Equation 6 for the (H^+) of minimum hydrolysis of atropine may be written in log form, thus

$$2 \log (H^+)_M = \log k_{OH^-} - \log k_{H^+} + \log K_w \quad \text{(Eq. 9)}$$

On substituting into Eq. 9 the log of the value of k_{H^+}, given in Eq. 8, and for $\log k_{OH^-}$ the log of the the constant reported previously (1) for the singly charged (protonated) form, i. e., $2.10 \times 10^{10} e^{-12,700/RT}$, the equation may then be expressed as

$$pH_M = {}^1/_2 \left(pK_w - 3.13 - \frac{983}{T} \right) \quad \text{(Eq. 10)}$$

The pH of minimum hydrolysis, Table II, changes inversely with increase in temperature, the values decreasing from 4.11 at 0° to 3.24 at 100°.

The half lives of solutions of atropine at various pH values and temperatures can be calculated for both hydroxyl-ion and hydrogen-ion catalysis. The former accounts for the instability above approximately pH 5 and the latter for instability below approximately pH 3. The half life at any hydrogen ion concentration between pH 3 and 5 is so great that its experimental determination is not practical. On the assumption that over this range of acidity instability of atropine is due to both hydroxyl ion and hydrogen ion catalyzed hydrolysis, and that the water reaction is minor, the half life at any pH value between these two regions and for any temperature can be calculated by means of the equation: $t_{1/2} = 0.693/(k_{H^+} + k_{OH^-})$. The logarithmic form of this equation, after setting k_{H^+} equal to $0.693/t_{1/2H^+}$ and k_{OH^-} equal to $0.693/t_{1/2OH^-}$ and simplifying, is $\log t_{1/2} = \log t_{1/2H^+} + \log t_{1/2OH^-} - \log (t_{1/2H^+} + t_{1/2OH^-})$. Calculated half lives of solutions of atropine over the region of pH 2 to 7 at temperatures from 20 to 100° are given in Table III.

Table I.—Experimental Half Lives of Atropine Compared to Calculated Values

Temp., °C.	N $HClO_4$	Half Life, Hr. Exptl.	Half Life, Hr. Calcd.
70.0	0.099	676	670
	0.199	352	333
	0.301	220	220
	0.401	161	166
80.0	0.099	329	328
	0.199	170	163
	0.301	107	108
	0.401	76	81
90.3	0.099	180	164
	0.199	80	81
	0.301	56	54
	0.401	37	40

Table II.—Stability of Atropine at pH of Minimum Hydrolysis

Temp., °C.	pH, min.	$t_{1/2}$[a] Yr.
0	4.11	3.17×10^4
10	3.97	7.50×10^3
20	3.84	1.96×10^3
30	3.74	5.87×10^2
40	3.64	1.88×10^2
50	3.54	6.34×10^1
60	3.46	2.36×10^1
70	3.39	9.42×10^0
80	3.32	3.93×10^0
90	3.28	1.82×10^0
100	3.24	8.8×10^{-1}

[a] $t_{1/2}$ at the pH of minimum hydrolysis was calculated by means of the following equations:

$$\frac{1}{t_{1/2}} = \frac{2k_{H^+}(H^+)_M}{0.693} =$$

$$2.886 \left(1.54 \times 10^7\ e^{-17,200/RT} \text{min.}^{-1}\right) (H^+)_M$$

$$-\log t_{1/2} = 7.6478 - \frac{3758.74}{T} + \log (H^+)_M$$

SUMMARY

The rate of acid hydrolysis of atropine has been measured in aqueous solution, and the activation energy and frequency factor calculated from Arrhenius plots. As expected, the hydrogen ion catalyzed hydrolysis of atropine is slow, the catalytic constant for the reaction at 40° having a value of 1.6×10^{-5} min.$^{-1}$ compared to that for the hydroxyl ion catalysis of the protonated species of atropine of 33.6 min.$^{-1}$.

Above pH 4.5, the predominant catalytic reaction involves hydroxyl ion; below pH 3, the predominant catalytic reaction involves hydrogen ion; while

Table III.—Predicted Stability of Atropine in Aqueous Solutions $t_{1/2}$ in Years

Temp., °C.	pH 7.0	6.0	5.0	4.5	4.0	3.0	2.0
20	2.7	27	266	811	1800	548	56
30	0.61	6.1	61	194	473	203	21
40	0.16	1.6	16	49	135	80.7	8.5
50	..	0.44	4.4	13.8	39.2	33.3	3.6
60	..	0.14	1.4	4.3	12.9	14.4	1.6
70	..	..	0.46	1.39	4.34	6.52	0.76
80	..	..	0.17	0.5	1.63	3.04	0.37
90	..	..	..	0.21	0.65	1.48	0.19
100	..	..	..	0.1	0.31	0.76	0.10

between these two regions, both ions contribute to the hydrolysis.

The pH for maximum stability of atropine in sterile aqueous solution varies between 4.11 at 0° and 3.24 at 100°.

REFERENCES

(1) Zvirblis, P., Socholitsky, I., and Kondritzer, A. A., THIS JOURNAL, **45**, 450(1956).
(2) "Dispensatory of the United States of America," 25th ed., 1955, J. B. Lippincott Co., Philadelphia, Pa., Part I, pp. 124-127.
(3) Manske, R. H. F., and Holmes, H. L., "The Alkaloids," Vol. I, Academic Press Inc., New York, 1950, p. 275.
(4) Kuhl, G., *Arch. expil. Path. Pharmakol.*, **109**, 295 (1925).
(5) Bodnar, J., and Ferenczy, J., *Arch. Pharm.*, **263**, 566(1925).
(6) Exler, Th., *Pharm. Weekblad*, **65**, 1152(1928).
(7) Schou, S. A., and Bjerregaard, P. B., *Dansk. Tidsskr. Farm.*, **6**, 185(1932).
(8) Vincke, E., and Delkers, H. A., *Med. Klin.* (*Munich*), **33**, 80(1937).
(9) Klyachkina, B. A., Guberman, P. F., and Lizgunova, M. V., *Farmatsiya*, No. 9–10, 19(1940).
(10) Blok, C. J., *Pharm. J.*, **155**, 282(1945).
(11) Ervenius, O., and Lindquist, H., *Farm. Revy.*, **45**, 577 (1946).
(12) Simon, I., *Gass. Ospedali Clin.* (Milan), **36**, 17(1915), cited from ref. (2). See also *J. Am. Med. Assoc.*, **64**, 705 (1915), and *C. A.*, **9**, 953⁸(1915).
(13) Bell, R. P., "Acid-Base Catalysis," Oxford University Press, 1941.
(14) Bell, R. P., "Advances in Catalysis," Vol. IV, Academic Press, Inc., New York, 1952.
(15) Kilpatrick, M., "Advances in Catalysis," Vol. VI, Academic Press, Inc., New York, 1954.
(16) Bullock, K., and Cannell, J. S., *Quart. J. Pharm. and Pharmacol.*, **14**, 241(1941).
(17) Glasstone, S., "Textbook of Physical Chemistry," 2nd ed., U. Van Nostrand Co., Inc., 1946, p. 1135.
(18) Snedecor, G. W., "Statistical Methods," 4th ed., The Iowa State College Press, Ames, Iowa, 1946, chap. 6.

Enzymatic Decomposition of Digitalis Glycosides*

By OLE GISVOLD† and S. E. WRIGHT‡

Using paper chromatographic methods the glycosidic constituents of freshly gathered leaves of *Digitalis lanata* have been examined before and after exposure to a limited period of enzymatic action. It appears that the original plant does not contain desglucoglycosides in quantities detectable by very sensitive methods, but that after exposure to enzyme degradation under defined conditions, glucose only is removed from the lanatosides to give acetyl digoxin, acetyl digitoxin, and probably acetyl gitoxin. The separation and identification of the less water soluble digitalis glycosides, some of their acetyl derivatives, and their genins, has been helped by the use of a new temperature-insensitive method of paper chromatography.

THE NUMBER of cardiac glycosides isolated from or detected in species of *Digitalis* continues to increase. Using paper chromatographic methods, several workers have detected apparently new glycosides in extracts of the leaves of *D. lanata and D. purpurea*. Jensen (1) detected nine unknown substances in an aqueous extract of *D. purpurea*. Sasakawa (2) revealed six substances which fluoresced similarly to the B series of digitalis glycosides, but the method used for extraction was not described. Aldrich, Frith, and Wright (3) used an enzyme-inhibiting, extraction process based on that used by Stoll and Kreis (4) in the isolation of the "genuine" glycosides lanatosides A, B, and C, and showed the presence of six substances, some of which were apparently cardioactive, in very young and mature plants of *D. lanata*.

Several new glycosides isolated from *D. lanata* and *D. purpurea* have been described in recent years, and the literature has been reviewed by Jensen (1) and by Tamm (5). In addition, we could mention gitaloxin (16 formyl gitoxin), verodoxin (16 formyl strospeside), and odoroside H (desgluco digitalinum verum), which have been reported by Haack, Kaiser, Gube, and Spingler (6) to be constituents of the "gitalin" fraction obtained from *D. purpurea*. Odoroside A also was detected in small quantities in *D. lanata* (7). Tschesche and co-workers (8) have isolated cardio-inactive compounds, resembling the digitalis glycosides in some of their chemical properties, from *D. lanata* and *D. purpurea* (digifolein) and from *D. purpurea* (digipurpurin). Murphy (9) found diginatin, the tentative structure of which is 16-hydroxy digoxin, occurring in small quantities with digoxin obtained from *D. lanata*. The same author (10) has also described another glycoside from *D. lanata*, gitoside, as the monodigitoxoside of gitoxigenin.

Since the work of Stoll and Kreis (4), it has been realized that many of the glycosides isolated from digitalis are artifacts produced during the

* Received March 27, 1957, from the University of Minnesota, College of Pharmacy, Minneapolis.
† Professor of Pharmaceutical Chemistry, College of Pharmacy, University of Minnesota.
‡ Associate Professor of Pharmacy, University of Sydney, Sydney, Australia.
Gratefulness is expressed for the laboratory hospitality of the University of Minnesota.
The authors wish to thank Professor R. Tschesche for digipurpurin, gitaloxin, and digifolein, and Dr. J. B. Murphy of Burroughs Wellcome and Co., Tuckahoe, N. Y., for diginatin and gitoside.

drying or extraction of the leaves, but a comparison of the glycosidic constituents of the plant before and after enzymatic activity has occurred does not appear to have been made. We have therefore attempted a paper chromatographic study of the constituents of mature leaves of *D. lanata* gathered freshly and extracted, as far as can be ascertained, by a method which prevents or reduces enzyme activity to a minimum, and compared them with the constituents present in an extract prepared in the same way, but after a brief period of enzyme action has occurred.

EXPERIMENTAL

Extraction

The leaves of *Digitalis lanata* were all collected six months after germination, and extracted within fifteen minutes of collection.

Enzyme Action Inhibited.—The process used was similar to that of Silberman and Thorp (11) based on the method of Stoll and Kreis (4). Twenty-four grams of the leaves were ground in a mortar with 12 Gm. of ammonium sulfate and 100 ml. of ethyl acetate added. The mixture then was shaken for four hours, the ethyl acetate poured off, and again shaken with two successive quantities of 50 cc. of ethyl acetate for one hour. The extract was filtered off by suction and the ethyl acetate removed *in vacuo* at a out 30 to 40°. The residue was adsorbed on kieselguhr and allowed to dry overnight in a wide dish. The kieselguhr mixture was then extracted with light petroleum in a Soxhlet until no more chlorophyll was removed, and then extracted by shaking with three successive portions of 30 cc. of chloroform: methanol 1:1. The chloroform-methanol extracts were combined and evaporated almost to dryness *in vacuo*. The extract was then adjusted to 8 cc. with alcohol 80%. This extract was then used for paper chromatography.

After Enzyme Action.—Twenty-four grams of leaves were disintegrated with 50 cc. of water in a Waring blendor, transferred to a beaker and the entire mash warmed at 50° to 60° in a water bath for 10 to 15 minutes, 50 Gm. of ammonium sulfate added and extracted with ethyl acetate as in the above process. Final adjustment to 8 cc. was made with 80 per cent alcohol, and this extract used for paper chromatography.

Paper Chromatography

Solvents used were: Ethyl acetate, washed with potassium carbonate and distilled; Benzene, thiophene free (Merck); Formamide, Fisher Reagent Chemical; Methyl isobutyl ketone redistilled, b. p. 117°; Isopropyl ether redistilled, b. p. 68–69°.

System 1.—Method of Silberman and Thorp (11) for more polar glycosides. Ethyl acetate–benzene—water, 84:16:50 (by volume). The solvents were shaken and allowed to separate overnight. The lower (aqueous) phase was placed in the bottom of a round glass tank, 20 cm. in diameter, 32 cm. high. The atmosphere in the tank was kept saturated by a sheet of filter paper lining the inside of the tank and dipping into the aqueous phase. To the upper (organic) phase, 2% of ethanol was added, then this

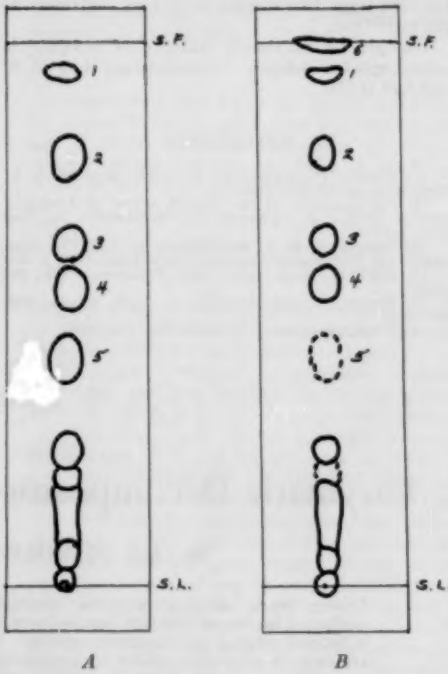


Fig. 1.—Paper chromatographs of extracts of *Digitalis lanata* (Solvent System 1 = ethyl acetate 84: benzene 16: water 50). *A*—Before enzymatic action; *B*—After enzymatic action; *1* and *2* = unknown substances; *3* = lanatoside *A*; *4* = lanatoside *B*; *5* = lanatoside *C*; *6* = desglucoglycosides; *S. F.* = solvent front; *S. L.* = starting line.

was placed in a glass dish resting on the bottom of the tank. After spotting, the paper was hung in the tank overnight to equilibrate, and then developed by the upward method for two-and-a-half to three hours, only, at 22 to 25°. Whatman No. 1 paper 12 cm. x 26 cm. was used and four spots of extract (0.01 cc. equivalent to 0.03 Gm. of the fresh plant) applied to each paper about 2 cm. from the bottom of the paper.

System 2.—For less water-soluble glycosides methyl isobutyl ketone-isopropyl ether, 100:25 (by volume), saturated with formamide, and allowed to separate clearly, was used. The nonpolar phase was placed in the bottom of the tank and used to develop the chromatograms by the upward method. The papers (Whatman No. 1) of the same size as in System 1 were passed slowly through a mixture of formamide-acetone, 30:70, and allowed to dry for about five minutes before spotting. The spots were applied as for Method 1, allowing about fifteen to twenty minutes between dipping the papers and developing. No period of equilibration was required, and the running time was from two-and-a-half to three hours.

The papers from System 2 were dried at 110° for twenty minutes before spraying. All papers were sprayed with 25% trichloroacetic acid in chloroform to which one drop of 100-vol. hydrogen peroxide

per 10 cc. had been added. They were then heated at 110° for ten minutes. Glycosides belonging to the A series (digitoxigenin derivatives) fluoresce yellow; B series (gitoxigenin derivatives) fluoresce bright blue; and those of the C series (digoxin derivatives) fluoresce pale blue in ultraviolet light after this treatment.

RESULTS

Paper Chromatography System 1.—In this system the desglucoglycosides digoxin, gitoxin, and digitoxin always follow the solvent front, but the lanatosides separate clearly. Extract A (enzyme action inhibited) showed only traces of material at the solvent front but lanatosides A, B, and C could be detected readily (Fig. 1–*A*). Two constituents giving a blue fluorescence in ultraviolet light after trichloroacetic acid treatment could be detected travelling ahead of lanatoside A (Fig. 1,*A*–1 and 2). The more water-soluble "L compounds" of Aldrich, *et al.* (3), also were present, but in the short running times used did not separate clearly. When diginatin, gitoside, and digifolein were run on this system no separation could be obtained, as they all followed the solvent front similar to the desglucoglycosides. Extract B (after enzyme action) showed a high concentration of glycosidic material at the solvent front (Fig. 1, *B*). The lanatosides also could be detected, but the spots were definitely less intense. The two unknown substances (Fig. 1, *A*—1 and 2) also could be detected in this extract.

Paper Chromatography System 2.—This was used to separate the less water-soluble glycosides. Extract A (enzyme action inhibited), when chromatographed on this system, showed only two spots running ahead of lanatoside A (Fig. 2, *A*–1 and 2). Both these fluoresced blue in ultraviolet light and

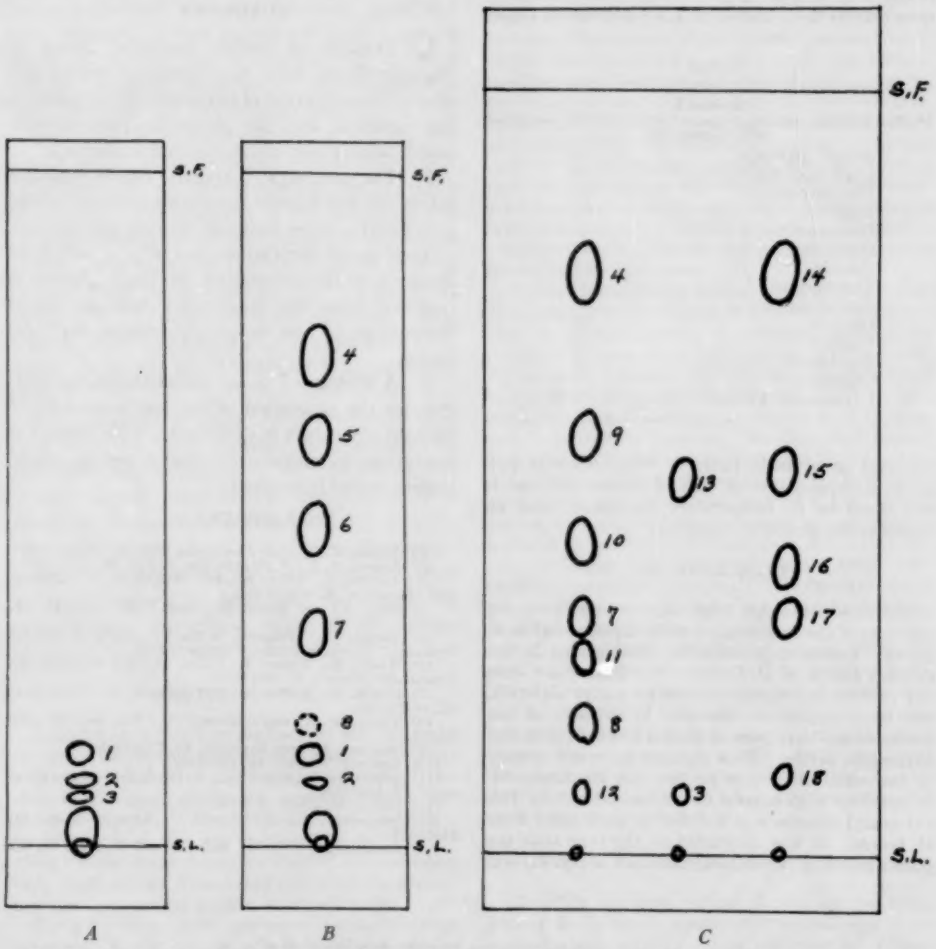


Fig. 2.—Paper chromatographs using Solvent System 2 (Methyl isobutyl ketone 100: isopropyl ether 25: formamide in formamide impregnated paper). *1* and *2* = unknown substances; *3* = lanatoside *A*; *4* = acetyl digitoxin; *5* and *6* = unknown substance; *7* = alpha acetyl digoxin; *8* = digoxin; *9* = digitoxin; *10* = gitoxigenin; *11* = gitoxin: *12* = diginatin; *13* = gitoside; *14* = digitoxigenin; *15* = digipurpurin; *16* = gitaloxin; *17* = digoxigenin; *18* = digifolein; *S. F.* = solvent front; *S. L.* = starting line.

were apparently the two substances which traveled between lanatoside A and the solvent front on System 1. As indicated by the control, these substances traveled behind digoxin (Fig. 2, *C*) and no trace of any desglucoglycoside could be detected in Extract A. When Extract B was chromatographed on this system a number of substances readily could be detected in front of lanatoside A (Fig. 2, *B*). These included the unknown substances seen in Extract A (Fig. 2, *A*–1 and 2) as well as acetyl digoxin, acetyl digitoxin, and two further unidentified blue fluorescing substances (Fig. 2, *A*–3 and 4) traveling between acetyl digitoxin and acetyl digoxin. This is the position in which α- and β-gitoxin would be expected to be found in this system, but no reference samples were available for checking. No digitoxin could be detected, but traces of digoxin were present (Fig. 2, *B*–8).

A number of glycosides derived from both *Digitalis lanata* and *Digitalis purpurea* also were chromatographed on this system (Fig. 2, *C*), and the R_f values

TABLE I

System 2 Methyl isobutyl ketone, isopropyl ether 100:25 saturated with formamide	
Acetyl digitoxin	75
Digitoxigenin	75
Digipurpurin	50
Digitoxin	53
Gitoxigenin	40
Gitaloxin	37
Gitoside	38
Digoxigenin	30
Acetyl digoxin-α	30
Gitoxin	25
Digoxin	15
Digifolein	10
Diginatin	7.5
Lanatoside A	7.5

obtained are given in Table I. The system is similar in its application to that of Kaiser (12) but is less sensitive to temperature variations, and no equilibration period is required.

CONCLUSIONS

The results indicate that there are no significant amounts of the desglucoglycosides digoxin, digitoxin, gitoxin, diginatin, gitoside, or their genins in the growing leaves of *D. lanata*. The fresh plant does not contain any acetyl digoxin or acetyl digitoxin, but these appear prominently in extracts of the leaves which have been subjected to a brief period of enzymatic action. This appears to result mainly in the removal of glucose but not deacetylation. In previous work carried out in this laboratory (13, 14) acetyl digoxin was isolated in good yield from *D. lanata*. It was concluded at the time that the plants grown in Minnesota contained acetyl digoxin as a "natural" glycoside (14). However, it is evident from this study that warming the fresh-leaf mash in the presence of water for only a brief period is sufficient to induce enzymatic breakdown of the principal glycosides. These were the conditions used previously (13). The effect of these conditions was confirmed by making a methyl isobutyl ketone extract of a fresh-leaf mash, after warming at 60° for fifteen minutes, and chromatographing the extract. The same qualitative chromatographic picture was obtained as for the ethyl acetate extract of the leaves made after enzymatic action.

It is apparent that the mixture of glycosides isolated from *D. lanata* will differ according to the method used to treat the leaves prior to extraction. Any mention of new constituents of digitalis plants should be accompanied by a detailed description of the method used to treat the leaves, and to make the extract, if the results are to be duplicated.

SUMMARY

1. Extracts of freshly gathered leaves of *Digitalis lanata* have been prepared before and after a limited period of enzymatic decomposition has occurred, and the glycosides present compared, using paper chromatographic methods.

2. The extracts prepared before enzyme action did not contain detectable amounts of the desglucoglycosides digitoxin, gitoxin, and digoxin, or their acetyl derivatives, but after a very brief exposure to the enzymes of the plant, glucose is removed from the lanatosides and the acetyl derivatives of the desglucoglycosides are produced.

3. A system of paper chromatography suitable for the separation of the less water-soluble digitalis glycosides is described. This system is insensitive to temperature change and no equilibration period is required.

REFERENCES

(1) Jensen, K. B., *Acta Pharmacol. Toxicol.*, **12**, 11(1956).
(2) Sasakawa, Y., *J. Pharm. Soc. Japan*, **75**, 946(1955).
(3) Aldrich, B., Frith, M., and Wright, S. E., *J. Pharm. and Pharmacol.*, **8**, 1042(1956).
(4) Stoll, A., and Kreis, W., *Helv. Chim. Acta*, **16**, 120 (1934).
(5) Tamm, C., "Progress in the Chemistry of Natural Products," Springer-Verlag, Vienna (1956).
(6) Haack, E., Kaiser, E., Gube, M., and Spingler, H., *Arzneimittel-Forsch.*, **6**, 176(1956).
(7) Haack, E., Kaiser, F., and Spingler, H., *Naturwiss.*, **42**, 441(1956).
(8) Tschesche, R., and Grimmer, G., *Chem. Ber.*, **68**, 1569 (1955).
(9) Murphy, J., THIS JOURNAL, **44**, 719(1955).
(10) Murphy, J., *ibid.*, **46**, 170(1957).
(11) Silberman, H., and Thorp, R. H., *J. Pharm. and Pharmacol.*, **5**, 438(1953).
(12) Kaiser, F., *Chem. Ber.*, **88**, 556(1955).
(13) Hopponen, R., and Gisvold, O., THIS JOURNAL, **41**, 146(1952).
(14) Krishnamurty, G. G., and Gisvold, O., *ibid.*, **41**, 152 (1952).

Antimicrobial Properties of a Series of β-Piperidinopropiophenones and Related Derivatives*

By H. J. FLORESTANO, M. E. BAHLER,† and A. D. CHANDLER

Of forty-two β-piperidinopropiophenones and related derivatives, ten possessed significant antimicrobial action, with relatively greater effectiveness indicated against fungi than against bacteria. Activity was increased as length of the alkyl chain in the para-position of the phenyl ring of β-piperidinopropiophenone was increased through the *n*-octyloxy substitution. Antibacterial activity was consistently reduced in the presence of serum; antifungal activity variably inhibited, and in some instances actual enhancement resulted. Data include comparison of antifungal activity of the most active derivatives with that of several known agents.

ALTHOUGH VARIOUSLY substituted ketones in general have been synthesized and tested for antimicrobial activity, relatively little has been reported in this regard for propiophenone members of this class of compounds. *In vitro* activity against *Cryptococcus neoformans* has been observed for isonitrosopropiophenone (1). Substitution of a dichloroacetyl group for the acetyl group of α-acetamido-β-hydroxy-*p*-nitropropiophenone, an intermediate in the synthesis of chloromycetin, resulted in the racemate of a dehydro derivative of the antibiotic for which activity was reported against *Candida albicans* (2) and *Trichomonas vaginalis* (3). On the other hand, certain propiophenones resulting from nuclear substitution of 2-aminophenol have been found to be devoid of antimicrobial properties (4).

Antispasmodic and anesthetic properties have been reported for a number of substituted β-piperidinopropiophenones (5, 6). It was of particular interest that similar compounds were found to possess antibacterial activity in addition (7). A series of 42 β-piperidinopropiophenones and related derivatives synthesized in our laboratory (8) have also been tested for antimicrobial properties, data on which are presented in this report.

EXPERIMENTAL

Activity of the various derivatives was tested by the serial twofold tube dilution method against the following organisms: *Staphylococcus aureus*, *Escherichia coli*, *Trichophyton mentagrophytes*, and *Candida albicans*. The synthetic broth described by Straus, *et al.* (9), was employed as the test medium for the staphylococcus, that of Sahyun, *et al.* (10), was used for *Escherichia coli*, while Sabouraud maltose broth served for the yeast and mold.

Stock solutions of the respective compounds were prepared, in most instances, in 95% ethyl alcohol at a concentration of 0.8%. Two of the derivatives (Nos. 1 and 15) were insoluble at this strength alcohol, but did dissolve in a 70% concentration; while a third compound (No. 28) was made up in acetone. Subsequent dilutions were prepared aseptically in tubes of broth so that final drug concentrations were contained in a volume of 5 cc. for the bacterial tests and in 10 cc. for the yeast and mold. Drugs were assayed in plain medium and in medium containing 10% normal horse serum. Although controls on the organic solvents were included in each series of tests, it was found in repeated determinations that the concentration of ethyl alcohol or acetone necessary to inhibit growth of the various organisms safely exceeded that present in the initial tube of the dilution series.

The inoculum for the bacteria consisted of 0.1 cc. of a suitably diluted eighteen-hour broth culture so that the initial number of cells averaged in the majority of cases about 1000 per cc. of drug broth. For *Candida albicans*, a thirty-hour broth culture was so diluted that the introduction of 0.1 cc. into each drug tube resulted in an inoculum of approximately 100,000 cells per cc. For the mold, growth appearing on Sabouraud maltose agar after fourteen days at 28° was transferred into flasks containing glass beads and Sabouraud maltose broth, shaken for ten minutes, and then strained through several layers of cheesecloth. The spores were counted on a hemocytometer and the suspension diluted with broth to contain approximately 10,000,000 spores per cc. Each serial-dilution tube received 0.1 cc. of the adjusted spore suspension.

Tubes inoculated with bacteria were incubated at 37° and examined at twenty-four-hour intervals. Those showing no growth at the end of twenty-four and forty-eight hours, respectively, were subcultured into fresh broth for determination of the minimum bactericidal concentrations for each organism. The yeast and mold tests were incubated at 28° with subcultures performed at seven and fourteen days for the lethal end points.

RESULTS

The data obtained indicated that certain members of this series of compounds possessed significant antimicrobial activity. The influence of various substitutions on *in vitro* efficacy will be apparent from the material presented in Table I. Essentially, the most active derivatives against the bacteria when tested in plain broth were the *n*-hexyloxy (No. 10),

* Received February 21, 1957, from the Research Department, Pitman-Moore Company, Division of Allied Laboratories, Inc., Indianapolis, Ind.

† Present address: Delco-Remy Division, General Motors Corp., Anderson, Ind.

TABLE I.—IN VITRO ANTIMICROBIAL ACTIVITY OF β-PIPERIDINOPROPIOPHENONES AND RELATED DERIVATIVES

No.	Compound Name	Minimum Microbicidal Concentration in mg. %							
		Staphylococcus aureus		*Escherichia coli*		*Trichophyton mentagrophytes*		*Candida albicans*	
		S[a]	NS	S	NS	S	NS	S	NS
1	*p*-Methoxy-β-piperidinopropiophenone·HCl	>50.0	50.0	100	25.0	25.0	25.0	>50.0	>50.0
2	*p*-Ethoxy-β-piperidinopropiophenone·HCl	>8.0	>8.0	>8.0	>8.0	25.0	>25.0	...	...
3	*p*-*n*-Propoxy-β-piperidinopropiophenone·HCl	...	...	...	...	...	...	>50.0	>50.0
4	*p*-Isopropoxy-β-piperidinopropiophenone·HCl	>16.0	>16.0	>16.0	>16.0	>50.0	>50.0	>50.0	>50.0
5	*p*-*n*-Butoxy-β-piperidinopropiophenone·HCl	25.0	12.5	>25.0	25.0	25.0	25.0	...	200
6	*p*-Isobutoxy-β-piperidinopropiophenone·HCl	...	...	...	...	...	...	>50.0	>50.0
7	*p*-sec-Butoxy-β-piperidinopropiophenone·HCl	...	...	...	...	...	...	>50.0	>50.0
8	*p*-*n*-Amoxy-β-piperidinopropiophenone·HCl	25.0	12.5	50.0	25.0	12.5	25.0	...	...
9	*p*-Isoamoxy-β-piperidinopropiophenone·HCl	...	...	...	...	...	...	50.0	50.0
10	*p*-*n*-Hexyloxy-β-piperidinopropiophenone·HCl	16.0	4.0	>16.0	8.0	25.0	12.5	...	...
11	*p*-*n*-Heptyloxy-β-piperidinopropiophenone·HCl	>8.0	2.0	>8.0	2.0	>25.0	12.5	...	...
12	*p*-*n*-Octyloxy-β-piperidinopropiophenone·HCl	>8.0	0.5	>8.0	1.0	>25.0	6.25	...	...
13	*p*-Benzyloxy-β-piperidinopropiophenone·HCl	>8.0	8.0	>8.0	>8.0	25.0	12.5	...	...
14	*p*-β-*n*-Butoxyethoxy-β-piperidinopropiophenone·HCl	>8.0	>8.0	>8.0	>8.0	>25.0	>25.0	...	...
15	*p*-β-Phenoxyethoxy-β-piperidinopropiophenone·HCl	>16.0	>16.0	>16.0	>16.0	50.0	50.0	>50.0	>50.0
16	2,4-Diethoxy-β-piperidinopropiophenone·HCl	>16.0	>16.0	>16.0	>16.0	50.0	50.0	>50.0	>50.0
17	2,5-Diethoxy-β-piperidinopropiophenone·HCl	>8.0	>8.0	>8.0	>8.0	25.0	>25.0	...	...
18	3,4-Diethoxy-β-piperidinopropiophenone·HCl	>8.0	>8.0	>8.0	>8.0	>25.0	>25.0	...	...
19	2,4-Di-*n*-butoxy-β-piperidinopropiophenone·HCl	>8.0	4.0	>8.0	8.0	25.0	25.0	...	...
20	2,5-Di-*n*-butoxy-β-piperidinopropiophenone·HCl	...	...	...	...	...	...	50.0	50.0
21	*o*-*n*-Butoxy-β-piperidinopropiophenone·HCl	>8.0	8.0	>8.0	>8.0	25.0	25.0	...	...
22	*p*-*n*-Butoxy-β-aminopropiophenone·HCl	>8.0	>8.0	>8.0	>8.0	>25.0	>25.0	...	...
23	*p*-*n*-Butoxy-β-dimethylaminopropiophenone·HCl	>8.0	4.0	>8.0	4.0	12.5	6.25	...	...
24	*p*-*n*-Butoxy-β-diethylaminopropiophenone·HCl	>8.0	4.0	>8.0	8.0	12.5	6.25	...	...
25	*p*-*n*-Butoxy-β-morpholinopropiophenone·HCl	>8.0	4.0	>8.0	4.0	6.25	0.78	>25.0	>25.0
26	*p*-*n*-Butoxy-β-phthalimidopropiophenone	>8.0	>8.0	>8.0	>8.0	>25.0	>25.0	...	...
27	*p*-*n*-Butyroxy-β-piperidinopropiophenone·HCl	...	...	...	...	...	...	>50.0	>50.0
28	*p*-*n*-Propoxy-γ-piperidino-*n*-butyrophenone·HCl	>25.0	>25.0	100	100	100	200	25.0	>50.0
29	*p*-*n*-Butoxy-β-piperidino-isobutyrophenone·HCl	>8.0	8.0	>8.0	>8.0	12.5	>25.0	...	...
30	*p*-*n*-Hexyloxy-α-phenyl-β-piperidinopropiophenone·HCl	>8.0	>8.0	>8.0	>8.0	>25.0	>25.0	...	...
31	α-Piperidinomethylcaprophenone·HCl	>8.0	>8.0	>8.0	>8.0	>25.0	>25.0	...	...
32	α-Ethyl-β-piperidinopropiophenone·HCl	>8.0	>8.0	>8.0	>8.0	>25.0	>25.0	...	...
33	1-Piperidino-2-phenyl-3-butanone·HCl	8.0	8.0	4.0	1.0	1.56	3.12	25.0	25.0
34	*p*-*n*-Butoxy-β-piperidinopropiophenone·HI	50.0	25.0	>50.0	25.0	...	...	...	...
35	*p*-*n*-Butoxy-β-piperidinopropiophenone·HBr	50.0	25.0	>50.0	25.0	...	...	...	...
36	*p*-*n*-Butoxy-β-piperidinopropiophenone·CH_3I	>25.0	12.5	>100	12.5	...	...	...	...
37	*p*-*n*-Butoxyphenyl-*n*-butyl-β-piperidinoethylcarbinol·HCl	>8.0	8.0	>8.0	8.0	12.5	>25.0	50.0	50.0
38	1-Piperidino-3-*p*-*n*-butoxyphenylpentene-2·HCl	>8.0	8.0	>8.0	8.0	12.5	>25.0	50.0	50.0
39	1-Piperidino-3-*p*-*n*-butoxyphenylheptene-2·HCl	...	2.0	...	4.0	6.25	6.25	12.5	12.5
40	3-Piperidino-1-(*p*-*n*-butoxyphenyl)propene-1·HCl	...	...	...	...	12.5	50.0	25.0	50.0
41	*p*-*n*-Butoxypropiophenone	>8.0	2.0	>8.0	>8.0	12.5	12.5	>50.0	>50.0
42	*p*-*n*-Butoxy-β-chloropropiophenone	>8.0	4.0	>8.0	4.0	12.5	0.78	>50.0	>50.0

S = broth with 10% normal horse serum; NS = plain broth.

TABLE II.—COMPARATIVE ACTIVITY OF SELECTED DERIVATIVES AGAINST VARIOUS FUNGI

No.	Compound Name	Minimum Fungicidal Concentration in mg. %											
		Trichophyton mentagrophytes		Trichophyton purpureum		Microsporum audouini		Microsporum lanosum		Candida albicans		Chaetomium globosum, NS	Sclerotinia fructicola, NS
		S[a]	NS	S	NS	S	NS	S	NS	S	NS		
25	*p*-*n*-Butoxy-β-morpholinopropiophenone·HCl	6.25	0.78	12.5	3.12	12.5	1.56	12.5	1.56	>25.0	>25.0	...	...
33	1-Piperidino-2-phenyl-3-butanone·HCl	1.56	3.12	3.12	3.12	1.56	0.78	6.25	6.25	25.0	25.0	25.0	3.12
39	1-Piperidino-3-*p*-*n*-butoxyphenylheptene-2·HCl	6.25	6.25	12.5	25.0	3.12	12.5	6.25	12.5	12.5	12.5	...	...
	Benzoic acid	>25.0	>25.0	>50.0	100	>50.0	25.0	>50.0	25.0	>50.0	>100	...	...
	Undecylenic acid	12.5	6.25	>25.0	6.25	12.5	3.12	>25.0	3.12	>25.0	25.0	...	...
	Asterol	12.5	>25.0	25.0	>25.0	12.5	>25.0	25.0	>25.0	>25.0	>25.0	2,000	2,000
	G-4	6.25	0.78	6.25	0.78	12.5	0.39	12.5	0.78	25.0	3.12	3.12	0.39

[a] S—broth with 10% normal horse serum; NS—plain broth.

n-heptyloxy (No. 11), *n*-octyloxy (No. 12), and 2,4-di-*n*-butoxy (No. 19) substituted β-piperidinopropiophenones; *p*-*n*-butoxy-β-dimethylaminopropiophenone (No. 23); *p*-*n*-butoxy-β-diethylaminopropiophenone (No. 24); *p*-*n*-butoxy-β-morpholinopropiophenone (No. 25); 1-piperidino-2-phenyl-3-butanone (No. 33); 1-piperidino-3-*p*-*n*-butoxyphenylheptene-2 (No. 39); and *p*-*n*-butoxy-β-chloropropiophenone (No. 42). With the exception of compound No. 28 against *Escherichia coli* and No. 33 against the staphylococcus, antibacterial action where demonstrated was diminished in the presence of serum.

Appreciable activity against *Trichophyton mentagrophytes* and *Candida albicans* was shown by members of the same group of compounds indicated above for the bacteria. Contrary to observations with the bacteria, however, antifungal action was not affected to the same degree by serum, and with certain derivatives efficacy appeared to be increased in its presence (Nos. 2, 8, 17, 28, 29, 33, 37, 38, and 40). Similar enhancement of activity by albumin has been reported for certain aliphatic β-diketones (11).

The antifungal activity exhibited by compounds No. 25, 33, and 39 seemed to warrant additional studies with these particular derivatives. They were therefore similarly tested against a wider spectrum of fungi, comparison being made with a group of known antifungal agents consisting of benzoic acid, undecylenic acid, Asterol,[1] and G-4.[2] As indicated in Table II, the ketones showed greater overall antifungal action, especially in the presence of serum, than did benzoic acid, undecylenic acid, and Asterol. Compared to G-4, however, their effectiveness in general was less in plain broth, but essentially equivalent to that of the bisphenol in serum broth.

DISCUSSION

While the series of compounds may not be sufficiently large to justify a detailed consideration of structure-activity relationship, several points of general interest are worthy of note, For example, antimicrobial activity of the β-piperidinopropiophenone derivatives was observed to increase as the length of the alkyl chain in the *para*-position of the phenyl ring increased. Duration of surface anesthetic effect of these derivatives also was found to increase with increase in length of the alkyl chain (6). However, whereas antimicrobial action increased through the *n*-octyloxy substitution, duration of anesthesia was abruptly decreased when *n*-octyloxy was substituted for *n*-heptyloxy.

In an investigation of a similar but smaller series of compounds studied primarily for their anesthetic properties, equal antibacterial activity was reported for *n*-propoxy- and isopropoxy-β-piperidinopropiophenone, while *n*-butoxy substitution resulted in greater activity than either the propoxy substitutions or *n*-isobutoxy substitution (7). Data presented here appear to confirm somewhat these findings.

Although possession of both antimicrobial and

[1] Trademark of Hoffmann-La Roche, Inc. for 2-dimethylamino-6 (β-diethylaminoethoxy)-benzothiazole dihydrochloride.

[2] Trademark of Sindar Corp. for bis(2-hydroxy-5-chlorophenyl)methane.

anesthetic properties of a drug is in no way unique, the fact that certain members of this series, i. e., the *n*-propoxy and *n*-butoxy substituted β-piperidinopropiophenone, have proved effective and safe as topical anesthetics makes their antimicrobial activity a decided advantage. Not only have pharmaceutical preparations containing *p*-*n*-butoxy-β-piperidinopropiophenone hydrochloride[1] shown self-sterilizing properties *per se*, but with the further incorporation of chlorobutanol definite synergistic action has been demonstrated against microorganisms commonly encountered in local infections (12).

Compound No. 33 has undergone limited clinical trial in the treatment of dermatophytoses. Results of experimental tests of its efficacy in the control of fungus growth on citrus fruit have indicated a potential usefulness in this field, a report of which will be forthcoming.

SUMMARY

1. A series of 42 β-piperidinopropiophenones and related derivatives were tested for antimicrobial activity.

2. Ten of the compounds showed significant action, with potentially stronger antifungal than antibacterial effectiveness being indicated.

3. Increasing alkyl-chain length in the *para*-position of the phenyl ring of β-piperidinopropiophenone resulted in increased activity through the *n*-octyloxy substitution.

4. Antibacterial activity was regularly antagonized by horse serum; antifungal activity variably so, with actual enhancement resulting in some instances.

5. The three most active of the derivatives studied possessed greater antifungal action than did benzoic acid, undecylenic acid, and Asterol; which were less active than G-4 in plain medium, but compared favorably with the bisphenol in the presence of horse serum.

[1] Solution Dyclone; Creme Dyclone, trademarks of Pitman-Moore Company.

REFERENCES

(1) Schmidt, E. G., Alvarez-De Choudens, J. A., McElvain, N. F., Beardsley, J., and Tawab, S. A. A., *Arch. Biochem.*, **26**, 15(1950).
(2) Long, L. M., and Troutman, H. D., *J. Am. Chem. Soc.*, **73**, 481(1951).
(3) Hamada, Y., *Osaka Daigaku Igaku Zasshi*, **5**, 437 (1953).
(4) Barber, M., and Haslewood, G. A. D., *Biochem. J.*, **39**, 285(1945).
(5) Denton, J. J., Turner, R. J., Neier, W. B., Lawson, V. A., and Schedl, H. P., *J. Am. Chem. Soc.*, **71**, 2048(1949).
(6) Abreau, B. E., Richards, A. B., Weaver, L. C., Burch, G. R., Bunde, C. A., Bockstahler, E. R., and Wright, D. L., *J. Pharmacol. Exptl. Therap.*, **115**, 419(1955).
(7) Profft, E., *Chem. Tech.* (Berlin), **5**, 13(1953).
(8) Bockstahler, E. R., and Wright, D. L., THIS JOURNAL, **46**, 542(1957).
(9) Straus, E., Dingle, J. H., and Finland, M., *J. Immunol.*, **42**, 331(1941).
(10) Sahyun, M., Beard, P., Schultz, E. W., Snow, J., and Cross, E., *J. Infectious Diseases*, **58**, 28(1936).
(11) Schales, O., *Arch. Biochem. and Biophys.*, **34**, 56 (1951).
(12) Florestano, H. J., and Bahler, M. E., THIS JOURNAL, **45**, 320(1956).

Alkoxy Derivatives of β-Piperidinopropiophenone and Related Compounds*

By EARL R. BOCKSTAHLER and DONALD L. WRIGHT

In a second investigation of the effect of alkoxy substituents on antispasmodic potency, a series of alkoxy derivatives of β-piperidinopropiophenone and some related structural variants were prepared. These showed no enhancement of antispasmodic potency over that of the parent compound, but some had noteworthy local anesthetic and antimicrobial activity. *p*-*n*-Butoxy-β-piperidinopropiophenone hydrochloride was selected as having most promise for drug use. It appears that, even though structural similarity may exist between some local anesthetics and antispasmodics, it is impossible to predict the effect of alkoxy substitution in one type of drug from its effect in the other.

IN A PREVIOUS communication (1), we suggested that the antispasmodic potency of a compound might be enhanced by substituting into it an alkoxy group of moderate size. This conjecture was based upon the effect of such substitution in a number of local anesthetics and on structural similarity of some local anesthetics and antispasmodics. A test series based on diethylaminoethyl benzilate gave inconclusive results. Antispasmodic potency did vary over a wide range as various alkoxy groups were introduced, but in no case was it greater than for the parent compound.

As a supplement, therefore, we undertook the

* Received February 21, 1957, from the Research Department, Pitman-Moore Co., Division of Allied Laboratories, Inc., Indianapolis, Ind.

TABLE I.—AMINOKETONE HYDROCHLORIDES

No.[a]	Substituents	Yield, %	M. p., °C.	Formula	Analysis[b] Calcd. % C	Calcd. % H	Found % C	Found % H
	A. β-Piperidinopropiophenone Hydrochlorides							
2	*p*-Ethoxy[c]	54	179.5–180.5	$C_{16}H_{23}NO_2 \cdot HCl$	64.54	8.12	64.86	8.12
3	*p*-*n*-Propoxy[c]	43	165–166	$C_{17}H_{25}NO_2 \cdot HCl$	65.49	8.41	65.90	7.96
4	*p*-Isopropoxy[c]	51	181.5–183	$C_{17}H_{25}NO_2 \cdot HCl$	65.49	8.41	65.70	8.13
5	*p*-*n*-Butoxy[c]	53	176–178	$C_{18}H_{27}NO_2 \cdot HCl$	66.34	8.66	66.26	8.60
6	*p*-Isobutoxy[c]	18	172–174	$C_{18}H_{27}NO_2 \cdot HCl$	66.34	8.66	66.61	8.40
7	*p*-sec-Butoxy	34	166–167	$C_{18}H_{27}NO_2 \cdot HCl$	66.34	8.66	66.40	8.43
8	*p*-*n*-Amoxy[c]	64	155.5–157	$C_{19}H_{29}NO_2 \cdot HCl$	67.14	8.90	66.93	8.41
9	*p*-Isoamoxy[c]	36	173–175	$C_{19}H_{29}NO_2 \cdot HCl$	67.14	8.90	67.55	8.72
10	*p*-*n*-Hexyloxy	28	153–154	$C_{20}H_{31}NO_2 \cdot HCl$	67.88	9.12	66.02	8.48
11	*p*-*n*-Heptyloxy	20	146–148	$C_{21}H_{33}NO_2 \cdot HCl$	68.53	9.31	68.43	9.07
12	*p*-*n*-Octyloxy	33	145–146	$C_{22}H_{35}NO_2 \cdot HCl$	69.16	9.50	68.96	9.29
13	*p*-Benzyloxy	48	165–167	$C_{21}H_{25}NO_2 \cdot HCl$	70.08	7.28	70.24	7.19
14	*p*-(β-*n*-Butoxy)ethoxy	27	134–136	$C_{20}H_{31}NO_3 \cdot HCl$	64.94	8.72	65.03	8.71
15	*p*-(β-Phenoxy)ethoxy	64	176–178	$C_{22}H_{27}NO_3 \cdot HCl$	67.76	7.23	68.05	7.32
16	2,4-Diethoxy	53	159–161	$C_{18}H_{27}NO_3 \cdot HCl$	63.24	8.25	63.08	8.23
17	2,5-Diethoxy	27	138.5–140	$C_{18}H_{27}NO_3 \cdot HCl$	63.24	8.25	63.41	8.44
18	3,4-Diethoxy	61	181–183	$C_{18}H_{27}NO_3 \cdot HCl$	63.24	8.25	63.46	8.04
19	2,4-Di-*n*-butoxy	60	155–157	$C_{22}H_{35}NO_3 \cdot HCl$	66.38	9.12	66.48	9.18
20	2,5-Di-*n*-butoxy	49	126–128	$C_{22}H_{35}NO_3 \cdot HCl$	66.38	9.12	65.99	8.99
	3,4-Di-*n*-butoxy	53	166–168	$C_{22}H_{35}NO_3 \cdot HCl$	66.38	9.12	66.51	9.11
21	*o*-*n*-Butoxy	67	132–134	$C_{18}H_{27}NO_2 \cdot HCl$	66.34	8.66	66.52	8.43
32	α-Ethyl	15	140–142	$C_{16}H_{23}NO \cdot HCl$	68.20	8.58	68.29	8.68
31	α-*n*-Butyl	18	147–149	$C_{18}H_{27}NO \cdot HCl$	69.77	9.11	69.88	9.21
29	*p*-*n*-Butoxy-α-methyl	65	170–172	$C_{19}H_{29}NO_2 \cdot HCl$	67.14	8.90	67.38	8.61
30	*p*-*n*-Hexyloxy-α-phenyl	42	136–137[d]	$C_{26}H_{35}NO_2 \cdot HCl$	72.63	8.64	72.75	8.30
27	*p*-Butyroxy[e]	35	174–174.5	$C_{19}H_{25}NO_3 \cdot HCl \cdot C_2H_5OH$	62.24	8.34	62.32	8.15
	B. *p*-*n*-Butoxypropiophenone Hydrochlorides							
22	β-Amino		168–170	$C_{13}H_{19}NO_2 \cdot HCl$	60.55	7.82	60.73	8.03
23	β-Dimethylamino	77	142–143	$C_{15}H_{23}NO_2 \cdot HCl$	63.03	8.46	63.07	7.98
	β-Methylamino	10	148–150	$C_{14}H_{21}NO_2 \cdot HCl$	61.85	8.16	62.21	8.34
24	β-Diethylamino	52	134.5–136	$C_{17}H_{27}NC_2 \cdot HCl$	65.05	8.99	65.05	8.57
25	β-Morpholino	45	168–170	$C_{17}H_{25}NO_3 \cdot HCl$	62.28	7.99	62.34	7.68
	C. *p*-*n*-Propoxy-γ-piperidinobutyrophenone Hydrochloride							
28			142–143	$C_{18}H_{27}NO_2 \cdot HCl$	66.34	8.66	66.53	8.92

[a] To facilitate comparison, the numbers used in the accompanying report by Florestano, Bahler, and Chandler on antimicrobial activities of many of these compounds, p. 540, are shown.
[b] Microanalyses by Oakwold Laboratories.
[c] Previously described by Profft (3).
[d] Free base m. 63–65°.
[e] Alcoholate.

synthesis of a second test series. The new series is based on a less potent parent, β-piperidinopropiophenone. This substance has been reported to have moderate antispasmodic activity *in vitro* (2). Its *p*-methoxy-, 3,4-dimethoxy, and 3,4-methylenedioxy-derivatives were also described, but showed little or no activity. This parallels our finding in the series of alkoxybenzilates that the methoxy derivative was by far the least potent.

In our new series, an alkoxy group was introduced in the *para* position of the benzene ring of β-piperidinopropiophenone, its size being varied from two to eight carbon atoms.[1] The compounds prepared are the first eleven listed in Table I. They were obtained by Mannich condensation of appropriately substituted acetophenones with formaldehyde and piperidine hydrochloride: $ROC_6H_4COCH_3 + HCHO + C_5H_{10}NH \cdot HCl \rightarrow ROC_6H_4COCH_2CH_2NC_5H_{10} \cdot HCl$.

Pharmacologic study of these compounds showed them to be potentially useful drugs. Accordingly, a number of exploratory structural modifications were tried: (*a*) Groups more elaborate than simple alkyl were utilized at the ether linkage. (*b*) The alkoxy group was attached at the *ortho* position rather than *para*. (*c*) A set of dialkoxy derivatives was prepared. (*d*) Alkyl groups were introduced on the α-carbon atom. (*e*) The alkoxy group was replaced by an ester grouping. (*f*) The piperidino radical was replaced by other amine radicals while holding the rest of the molecule constant. (*g*) The chain linking carbonyl to piperidino was lengthened to three carbon atoms. The compounds illustrating these modifications are listed in the remainder of Table I.

[1] These compounds are the subject matter of U. S. Patent 2,771,391, issued November 20, 1956, to one of the authors.

TABLE II.—INTERMEDIATE KETONES RC_6H_4COR'

R	R'	Method of Preparation	Yield, %	M. p., °C.	B. p., °C./mm Hg	Formula	Analysis[a] Calcd. % C	Calcd. % H	Found % C	Found % H
p-sec-Butoxy	Methyl	A	54	...	119–120/3	$C_{13}H_{18}O_2$	74.97	8.39	75.02	8.27
p-*n*-Heptyloxy	Methyl	A	60	...	164–166/3	$C_{15}H_{22}O_2$	76.88	9.47	76.96	9.50
p-(β-*n*-Butoxyethoxy	Methyl	A	30	...	150–152/4	$C_{14}H_{20}O_3$	71.14	8.53	71.23	8.62
p-(β-Phenoxyethoxy	Methyl	A	38	141–142.5	...	$C_{16}H_{16}O_3$	74.99	6.29	74.88	6.25
3,4-Di-*n*-butoxy	Methyl	B	37	42–44	158–160/5	$C_{16}H_{24}O_3$	72.69	9.15	72.84	9.15
o-*n*-Butoxy	Methyl	A	81	...	119–120/3	$C_{13}H_{18}O_2$	74.97	8.39	75.12	8.57
p-*n*-Butoxy	β-Chloroethyl	B	28	51–52	...	$C_{14}H_{19}O_2Cl$	64.85	7.12	65.16	7.35
p-Butyroxy	Methyl	C	74	37–38	141/3	$C_{12}H_{16}O_3$	69.88	6.84	69.40	6.82

[a] Microanalyses by Oakwold Laboratories.

In general, the method of synthesis remained the same, i. e., Mannich condensation of suitably substituted acetophenones. Two exceptions were necessary. Gabriel's phthalimide synthesis was used in preparing the primary amine under (*f*). The Mannich reaction would have required the use of ammonium chloride, which usually gives a mixture of products difficult to separate. The three-carbon chain of (*g*), impossible by the Mannich route, was obtained by reaction of a Grignard reagent with a nitrile: $p\text{-}CH_3CH_2CH_2OC_6H_4MgBr + CNCH_2CH_2CH_2NC_5H_{10} \rightarrow p\text{-}CH_3CH_2CH_2OC_6H_4COCH_2CH_2CH_2NC_5H_{10}$.

Profft (3) has described a series of compounds similar to ours and in some cases identical, out of which he developed the local anesthetic "Falicaine," *p*-*n*-propoxy-β-piperidinopropiophenone hydrochloride. Except for a few of the variants, our syntheses were completed before Profft's reports appeared. Considering the similarity in concepts involved, there was surprisingly little duplication of effort in the two independent investigations. Both laboratories made the simple monoalkoxy compounds, normal and iso, up to amoxy. They then diverged, there being no duplication beyond this point.

EXPERIMENTAL

Intermediate Ketones.—Several of the intermediate ketones used in our syntheses have not been described before. These are listed in Table II. They were prepared by the following methods: (A) *p*- or *o*-hydroxyacetophenone was refluxed in alcohol solution with the appropriate alkyl bromide in the presence of potassium carbonate; (B) catechol di-*n*-butyl ether was reacted with acetyl chloride, phenyl butyl ether with β-chloropropionyl chloride, under Friedel-Crafts conditions (aluminum chloride catalyst in carbon disulfide); and (C) *p*-hydroxyacetophenone was refluxed with butyric anhydride.

β-Piperidinopropiophenone Hydrochlorides.—Initial attempts to prepare these compounds, using conditions conventional for the Mannich reaction, gave erratic results. The modification recommended by Winstein and co-workers (4), in which nitromethane was used as solvent, proved to be much more dependable, and was adopted for the series. The morpholino-, dialkylamino-, and methylaminopropiophenones were made in the same way.

In a typical run, a mixture of 28.7 Gm. of *p*-ethoxyacetophenone, 36.3 Gm. of piperidine hydrochloride, 13.5 Gm. of paraformaldehyde, 0.75 ml. of concentrated hydrochloric acid, 157.5 ml. of nitromethane, 22.5 ml. of ethanol, and 45 ml. of toluene was refluxed for one hour, removing water formed in the reaction by means of a condensate trap. The mixture was then cooled and allowed to stand. The crystals which formed were collected by filtration and recrystallized from methyl ethyl ketone.

Nitroethane was substituted for nitromethane in making the *p*-*n*-butoxy and *p*-*n*-octyloxy derivatives, with no apparent difference in results.

The products are white, crystalline substances, stable in the dry state, but decomposed when heated in aqueous solution. The *n*-hexyloxy derivative seemed especially labile. It was never obtained analytically pure, always being contaminated with a little piperidine hydrochloride, presumably formed by cleavage during recrystallization.

***p*-*n*-Butoxy-β-phthalimidopropiophenone.**—A solution of 34 Gm. of *p*-*n*-butoxy-β-chloropropiophenone in 50 ml. of xylene was added dropwise to a boiling suspension of 39 Gm. of potassium phthalimide in 100 ml. of xylene, mechanically stirred. The mixture was boiled and stirred for one and a half hours after addition was completed, then filtered hot, and chilled in an ice bath. The crystals which formed were collected by filtration and recrystallized from alcohol. Yield, 28 Gm.; m. p. 102–103°.

Anal.—Calcd. for $C_{21}H_{21}NO_4$: 71.77% C; 6.02% H. Found: 72.05% C; 6.10% H.

***p*-*n*-Butoxy-β-aminopropiophenone Hydrochloride.**—A mixture of 20 Gm. of *p*-*n*-butoxy-β-phthalimidopropiophenone, 75 ml. of glacial acetic acid, and 55 ml. of concentrated hydrochloric acid was refluxed for twenty hours. The mixture was then diluted with an equal volume of water, boiled half an hour with decolorizing carbon and filtered hot, then evaporated to near dryness on the steam bath. The residue was stirred well with 100 ml. of chloroform and filtered, to remove phthalic acid. The filtrate was evaporated to dryness by means of a stream of air at room temperature. The solid residue was recrystallized from a mixture of methanol and ethyl acetate. Yield, 5 Gm.

***p*-*n*-Propoxy-γ-piperidinobutyrophenone Hydrochloride.**—The procedure of Humphlett, Weiss, and Hauser (5) for making γ-diethylaminopropyl ketones was followed. The yield from 27 Gm. of γ-piperidinobutyronitrile and 43 Gm. of *p*-bromophenyl *n*-propyl ether was 22 Gm. The free base boiled at 194–195° at 3 mm.

DISCUSSION

Pharmacologic study of these compounds (6) showed them to be of interest, although not in the anticipated sense. They were without merit as atropine or papaverine-like antispasmodics. They did, however, have the special ability to relieve the signs of a canine tremor-rigidity syndrome, which simulates Parkinson's Disease. The *p*-*n*-butoxy derivative, which was the most potent in this respect, was submitted for human clinical trial against Parkinsonism, but proved to be ineffective (7).

The series continued to be of interest, however, in that the compounds were found to be potent local anesthetics. The *p*-*n*-butoxy derivative was again singled out for human trial. A number of clinical studies have been made with favorable results (8), and the compound is now being marketed under the generic name dyclonine hydrochloride.[2]

None of the exploratory structural variants was found to have any pharmacological advantage over the group of simple *p*-alkoxy derivatives.

Bacteriological study revealed that compounds of this series have significant antimicrobial action *in vitro* (9).

The supposition on which these investigations was based, that it might be possible to enhance antispasmodic potency by introducing alkoxy groups, is therefore not substantiated. Instead, the two series studied have returned to the starting point by showing once more that such substitution can enhance local anesthetic activity.

[2] Pitman-Moore Company brand name: Dyclone.

REFERENCES

(1) Bockstahler, E. R., and Wright, D. L., *J. Am. Chem. Soc.*, **71**, 3760(1949).
(2) Denton, J. J., Turner, R. J., Neier, W. B., Lawson, V. A., and Schedl, H. P., *ibid.*, **71**, 2048(1949).
(3) Profft, E., *Chem. Tech. (Berlin)*, **3**, 210(1951); **4**, 241 (1952); **5**, 13(1953).
(4) Winstein, S., Jacobs, T. L., Seymour, D., and Linden, G. B., *J. Org. Chem.*, **11**, 215(1946).
(5) Humphlett, W. J., Weiss, M. J., and Hauser, C. R., *J. Am. Chem. Soc.*, **70**, 4020(1948).
(6) Abreu, B. E., Richards, A. B., Weaver, L. C., Burch, G. R., Bunde, C. A., Bockstahler, E. R., and Wright, D. L., *J. Pharmacol. Exptl. Therap.*, **115**, 419(1955).
(7) Schwab, R. S., Personal communication.
(8) Shelmire, B., Gastineau, F. M., and Shields, T. L., *Am. Med. Assoc., Arch. Dermatol.*, **71**, 728(1955). Arora, R. B., Consul, B. N., Sharma, V. N., and Kulshrestha, P. P., *Eye, Ear, Nose, Throat Monthly*, **34**, 593(1955). Fisher, H. E., *Missouri Medicine*, **52**, 943(1955).
(9) Florestano, H. J., and Bahler, M. E., THIS JOURNAL, **45**, 320(1956). Florestano, H. J., Bahler, M. E., and Chandler, A. D., *ibid.*, **46**, 539(1957).

A Comparative Human *In Vivo* Study of Antacids*

By E. W. PACKMAN, D. D. ABBOTT, J. I. FEINMAN, and J. W. E. HARRISSON

The antacid effectiveness of: dihydroxy aluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate in humans has been compared. Samples of gastric contents were removed over a seventy-five-minute period and evaluated for pH and free and total acidity. The usefulness of the four antacids, based upon controlled pH in the order of their decreasing effectiveness, was as follows: dihydroxy aluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate

EVALUATION of the true efficacy of potential antacid agents is a tedious task. Information may be obtained from *in vitro* measurements as described by the U. S. P. or by the method described by Holbert and his associates (1); however, there are many important factors which cannot be investigated by *in vitro* tests. Such factors include: effect upon digestive processes, effect, if any, in producing acid-rebound, effect upon normal gastrointestinal function (diarrhea or constipation), and effect upon gastric mucosa (does it decrease or increase irritation).

A biologic approach is necessary to investigate any or all of the above factors. Kirsner and his associates (2) reviewed the currently used gastric antacids and concluded that *in vitro* measurements are no substitute for the direct biologic evaluation of an antacid.

In previous studies we have studied the antacid properties of dihydroxy aluminum sodium carbonate (DASC), aluminum hydroxide, cal-

* Received May 2, 1957, from the LaWall and Harrisson Research Laboratories, 1921 Walnut Street, Philadelphia 3, Pa.
Presented to the Scientific Section, A. PH. A., New York meeting, May 1956.
The study was made under a research grant by the American Chicle Company, New York, which we acknowledge as well as the enthusiastic support of DR. ROBERT HEGGIE, Director of Research.

cium carbonate, and sodium bicarbonate in the Pavlov fistula dog (3) and in guinea pigs (4, 5). This latter investigation dealt with the prevention or alteration of histamine induced ulceration in the guinea pig.

Each of these studies has clearly indicated the superior antacid properties of certain antacids over the other agents that were studied. It has been shown that biologic experiments are more conclusive than *in vitro* tests for antacids. However, in the final analysis after all pertinent information concerning an antacid has been obtained from animals, it is desirable to conduct human clinical studies to determine the effectiveness of an antacid in the physiologic surroundings in which they are meant to work.

Our present investigation has considered the comparative antacid effectiveness of dihydroxy aluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate, in human subjects.

EXPERIMENTAL DESIGN

Selection of Subjects.—Healthy, adult males who secreted gastric juice with a high concentration of acid, were fasted for eighteen hours then intubated via the nose with a standard Levine tube. This tube was left *in situ* during the study. An initial gastric sample (3 ml.) was withdrawn with the aid of a special glass syringe. Measurement of pH was made upon this sample, and only subjects whose gastric pH was 2.5 or below were selected for the study.

Administration of Antacids.—One gram of the antacid, as 5 ml. of an aqueous suspension or solution, was administered via the intubation tube after the initial gastric sampling.

Gastric Sampling.—After the zero or initial gastric sampling and following antacid administration, 3-ml. samples of gastric juice were asperated via the intubation tube every fifteen minutes, for a seventy-five-minute period, each sample of gastric juice was examined for free and total titratable acidity employing Topffer's reagent and phenolphthalein as indicators. The determination of pH was made with a Beckman pH meter.

PRELIMINARY EXPERIMENTS

Histamine as a Stimulant.—After intubation for gastric sampling, a subcutaneous injection of 0.5 mg. histamine acid phosphate was made into the forearm of the subject. Gastric samples were taken at fifteen-minute intervals over a seventy-five-minute period.

The gastric secretory response to histamine was found not to be uniform and no correlation could be obtained between the response of an individual upon different days. Furthermore, several of the subjects became sensitive to histamine and therefore, the use of histamine was discontinued.

Alcohol as a Stimulant.—Fifty ml. of 30% ethyl alcohol was administered via intubation. Response among patients was not uniform from day to day, hence the use of alcohol as a stimulant was discontinued.

Caffeine as a Stimulant.—The results obtained

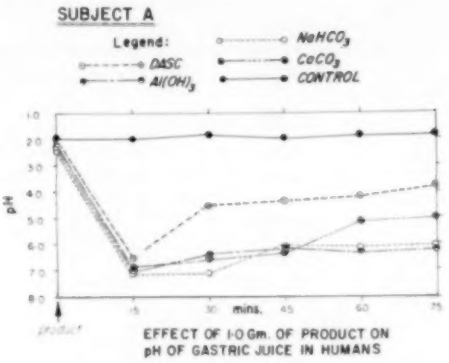


Figure 1.

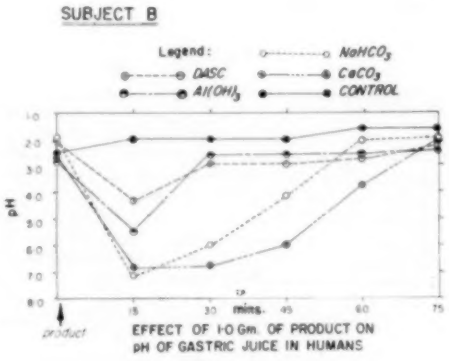


Figure 2.

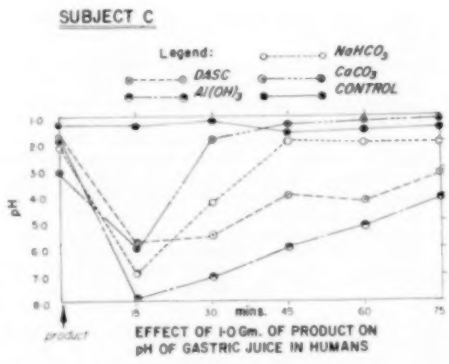


Figure 3.

with caffeine were similar to those observed with alcohol, therefore caffeine was also discontinued.

As it appeared desirable to employ subjects who produced a reasonably consistent and high gastric acid value, a different approach was attempted.

After examining many subjects, several were found who produced a copious flow of gastric acid without stimulation. These "good acid producers" were chosen as subjects for our study. A time lapse of at least one week was maintained between individual experiments.

RESULTS

Subject A (Fig. 1).—An immediate rise in pH to pH 6–7 was evidenced at the fifteen-minute sampling following the administration of 1 Gm. of DASC; thereafter a pH range of from 3 to 5 was maintained throughout the seventy-five-minute experimental period. An immediate rise in pH to pH 6–7 was produced by 1 Gm. of aluminum hydroxide at the fifteen-minute sampling and the pH remained between 6 and 7 throughout the experimental period. One gram of sodium bicarbonate caused an immediate rise in pH to pH 7–8 at the fifteen minute sampling and the pH remained between 6 and 7 throughout the experimental period. With calcium carbonate, 1 Gm., an immediate rise in pH to pH 6–7 was evidenced at the fifteen-minute sampling, thereafter a pH range of from 5.5 to 6.7 was maintained throughout the experimental period. A pH of approximately 2 was maintained throughout the observation period when the subject received no antacid. Little or no free titratable acidity was evident at pH 3 or above.

Subject B (Fig. 2).—A pH range of 3 to 5 was maintained throughout the seventy-five-minute experimental period by a dose of 1 Gm. of DASC. Aluminum hydroxide, 1 Gm., produced an immediate rise in pH to pH 5–6 at the fifteen-minute sampling, but thereafter the pH was between 2 and 3 throughout the experimental period. A pH of 6–7 was evidenced during the initial forty-five minutes following the administration of 1 Gm. of sodium bicarbonate; thereafter there was a sharp decrease to approximately pH 2.5. One gram of calcium carbonate caused an immediate rise in pH to pH 7–8 at the fifteen-minute sampling; thereafter there was a sharp decrease to approximately pH 2.5. A pH of approximately 2 was maintained throughout the observation period when the subject received no antacid. Little or no free titratable acidity was evident at pH 3 or above.

Subject C (Fig. 3).—Following the ingestion of 1 Gm. of DASC an immediate rise in pH to pH 5–6 was evidenced at the fifteen-minute sampling, thereafter a pH range from 3 to 5 was maintained throughout the seventy-five-minute experimental period. Aluminum hydroxide, 1 Gm., produced an immediate rise in pH to pH 7–8 at the fifteen-minute sampling, thereafter the pH remained between 5–6 until the seventy-five minute sample when the pH returned to 3. One gram of sodium bicarbonate effected an immediate rise in pH to pH 6–7 at the fifteen-minute sampling; thereafter there was a sharp decrease to approximately pH 2. An immediate rise in pH to pH 6–7 was produced by 1 Gm. of calcium carbonate at the fifteen-minute sampling; thereafter there was a sharp decrease to approximately pH 1.5. A pH of approximately 2 was maintained throughout the observation period when the subject received no antacid. Little or no free titratable acidity was evident at pH 3 or above.

Subject D (Fig. 4).—A pH between 5 and 6 was maintained during the first forty-five minutes by 1 Gm. of DASC; thereafter the pH remained at approximately 3.5. Aluminum hydroxide, 1 Gm., caused an immediate rise in pH 5–6 at the fifteen-minute sampling, thereafter there was a sharp decrease to approximately pH 1.5. With sodium bicarbonate, 1 Gm., there was an immediate rise to pH 6–7 at the fifteen-minute sampling; thereafter there was a sharp decrease to approximately pH 2. A pH of 5–6 was maintained throughout the experimental period by 1 Gm. of calcium carbonate. A pH of approximately 1.5 was maintained throughout the observation period when the subject received no antacid. Little or no free titratable acidity was evident at pH 3 or above.

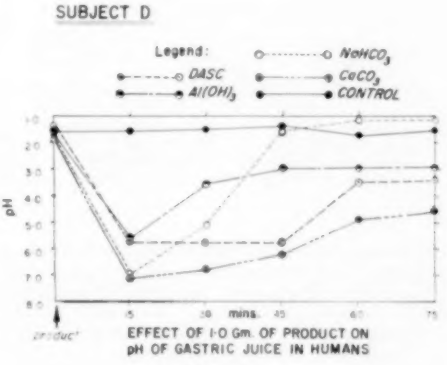


Figure 4.

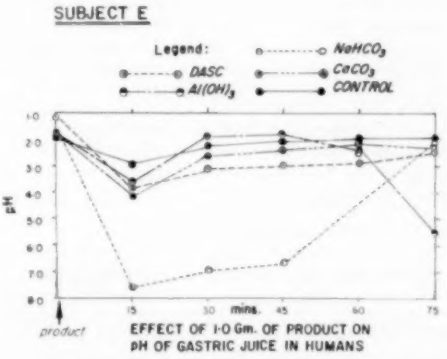


Figure 5.

Subject E (Fig. 5).—A pH between 3 and 4 was maintained by 1 Gm. of DASC during the first sixty minutes, at the final sampling (seventy-five minutes) the pH was approximately 2.5. With one gram of aluminum hydroxide an initial pH between

3 and 4 was evidenced at the fifteen-minute sampling, during the next forty-five minutes the pH was approximately 2, at the final sampling (seventy-five minutes) the pH was 5.5. A pH between 7 and 8 was maintained by 1 Gm. of sodium bicarbonate for the first forty-five minutes, thereafter there was a sharp decrease to pH 2.5. An initial pH of 3–4 was evidenced, after the administration of 1 Gm. of calcium carbonate, at the fifteen-minute sampling; thereafter the pH remained between 2 and 3. A pH of approximately 2.5 was maintained throughout the observation period when the subject received no antacid. Little or no free titratable acidity was evident at pH 3 or above.

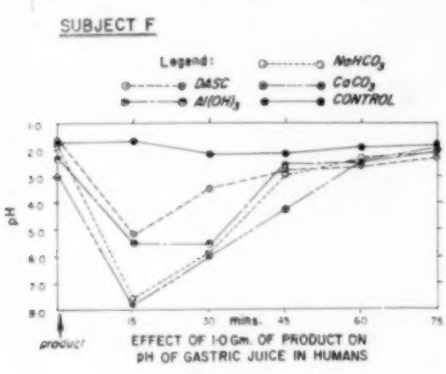


Figure 6.

Subject F (Fig. 6).—An initial pH rise to 5–6 was evidenced after the ingestion of 1 Gm. of DASC at the fifteen-minute sampling, thereafter a pH between 3 and 4 was maintained throughout the experimental period. One gram of aluminum hydroxide produced a pH of 5–6 during the first thirty minutes, thereafter a pH between 2 and 3 was maintained throughout the experimental period. Sodium bicarbonate, 1 Gm., caused an immediate rise in pH to pH 7–8 at the fifteen-minute sampling, thereafter there was a sharp decrease to pH 2. An immediate rise in pH to pH 7–8 was evidenced after the administration of 1 Gm. of calcium carbonate at the fifteen-minute sampling, thereafter there was a sharp decrease to pH 2. A pH of approximately 2 was maintained throughout the observation period when the subject received no antacid. Little or no free titratable acidity was evident at pH 3 or above.

DISCUSSION

Evaluation of the efficacy of an antacid is usually based on the neutralization or reduction of the free titratable acid concentration of the gastric juice and the ability of the antacid to maintain the pH of the gastric contents within limits approximating pH 3 to 5.5. Further reduction in hydrogen ion concentration could excessively inhibit the physiologic digestive processes of the stomach.

It has been the opinion of many gastroenterologists that the measurement of free titratable acidity of the gastric juice does not parallel the proved efficacy of established products; moreover, they have stated that the measurement of pH is often more useful in determining the therapeutic effectiveness of medicinal agents, upon gastric secretions.

Results of this study comparing four well-known antacid agents are clearly indicative of the validity of employing pH as the criterion of antacid effectiveness; since all the antacids studied reduced the free titratable acidity of the gastric juice to a very low level; yet, this reduction does not always reflect the pH. For example, one subject had a low level of free titratable acidity when the pH of the gastric contents was 2.5, while a second subject exhibited a higher free acidity level at the same pH. Although we have in this study determined both free and combined titratable acidity in addition to pH, we here confine our comparison of the antacids to data upon the pH of the gastric secretions following administration of these agents.

Each of the six subjects employed in this study may be classified as a "good acid producer." The "resting pH" of these individuals approximates pH 1.5 to 2 and remained essentially at this level throughout the seventy-five minute period, when no antacid agent was administered. In nearly all the subjects studied one gram of DASC maintained the pH of the gastric juice within or close to the optimum range of pH 3 to 5.5 over the entire observation period. Aluminum hydroxide, sodium bicarbonate, and calcium carbonate at a dose of one Gm., all raised the pH above the desired limits initially and maintained it above the optimum level of 5.5 or the pH fell below 3.0 within the experimental period.

These results are in close agreement with the data obtained when these same four antacid agents were evaluated in the gastric fistula dog. A study upon humans as presented here makes use of the physiologic surroundings in which antacid agents exert their effects, namely the healthy human stomach. Moreover, in this study the absence of any chemical stimulant, i. e., histamine, caffeine, or alcohol, also duplicates true physiologic conditions. From the data which we have obtained in this human clinical study, the usefulness of the four antacids based upon controlled pH, in the order of their decreasing effectiveness were dihydroxy aluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate.

CONCLUSIONS

1. Four well-known antacids have been compared in human subjects by removing samples of gastric juice at specified intervals.

2. In a dosage of 1.0 Gm. the antacids in the order of their decreasing effectiveness based upon maintenance of pH between 3 and 5, and duration of neutralization were: dihydroxy aluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate.

3. Over a seventy-five-minute observation period, only DASC maintained the gastric pH within or close to the optimum range of pH 3 to pH 5.5.

4. The advantages of a human clinical study for evaluating potential antacid agents are presented.

REFERENCES

(1) Holbert, J. M., Noble, N., and Grote, I. W., THIS JOURNAL, **37**, 292(1948).
(2) Kirsner, J. B., Palmer, W. L., Levin, E., and Klotz, A. P., *Ann. Internal Med.*, **35**, 785(1951).
(3) Packman, E. W., Goldberg, M. E., and Harrisson, J. W. E., THIS JOURNAL, **45**, 735(1956).
(4) Harrisson, J. W. E., Packman, E. W., Trabin, B., and Goldberg, M. W., *ibid.*, **45**, 738(1956).
(5) Harrisson, J. W. E., Packman, E. W., Trabin, B., and Goldberg, M. E., *J. Exptl. Med. Surg.* In Press.

Comparative *In Vivo* Methods for Evaluating Antacids in Humans*

By JOSEPH W. E. HARRISSON, D. D. ABBOTT, J. I. FEINMAN, and E. W. PACKMAN

A continuous pH recording device is described whereby gastric acidity may be determined accurately without removal of gastric juice from the stomach. The instrument can be assembled from equipment readily available commercially. By use of the instrument described, four compounds have been tested for their efficiency as antacids. These antacids, listed in decreasing order of effectiveness, are dihydroxy aluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate.

WHEN EVALUATING the true efficacy of a potential antacid agent, there is no substitute for *in vivo* tests. Many investigators have studied the effectiveness of antacids in Pavlov pouch or fistula dogs, or in the pylorus ligated rat or by inhibiting histamine induced ulceration in the guinea pig. In the final analysis, however, after all pertinent information has been obtained from animals, it is desirable to conduct human clinical studies.

For the most part, clinical studies have been performed by passing a Levine tube into the stomach and removing samples of gastric contents by frequent aspirations. These samples were then titrated against 0.1 *N* sodium hydroxide with Topfers reagent, or phenolphthalein as indicators and the results expressed as free hydrochloric acid or total acid respectively.

Disadvantages of this multi-sampling procedure were soon recognized as follows: Removal of samples of gastric contents over a period of time markedly reduced the volume of acid remaining in the stomach. Frequent sampling also reduced the quantity of antacid agent which could react in the stomach. The samples varied as to their mucous content, thus the acid determinations varied greatly. Frequent aspirations from the stomach could in themselves stimulate either acid or mucous production.

To overcome these apparent difficulties, it seemed necessary to obtain a measure of gastric acidity without removing samples from the stomach. Flexner and Kniazak (1, 2) prepared a glass electrode which could be swallowed by a human. With this electrode connected to a pH meter, a continuous reading of gastric pH would be made without removing samples of gastric contents. Later Rossett and Flexner (3) employing this method of continuous recording of gastric pH studied the effects of various antacids *in situ*. More recently Rovelstad (4) and Rovelstad, Owen and Magath (5) employed the continuous recording of pH to study gastric and duodenal contents of patients with and without duodenal ulcers. The latter authors also reported on the factors influencing the continuous recording of gastric and duodenal pH.

This communication is concerned with the description of a continuous pH recording method of our own design using commercially available equipment.

For purposes of comparison intermittent samplings of gastric contents were taken from the subjects. The relative effectiveness of four antacid agents was studied by this procedure (6).

* Received May 2, 1957, from LaWall and Harrisson Research Laboratories, 1921 Walnut Street, Philadelphia 3, Pa.
Presented to the Scientific Section, A. PH. A., New York meeting, April–May, 1957.
The study was made under a research grant by the American Chicle Co., New York, which we acknowledge as well as the enthusiastic support of Dr. Robert Heggie, Director of Research.

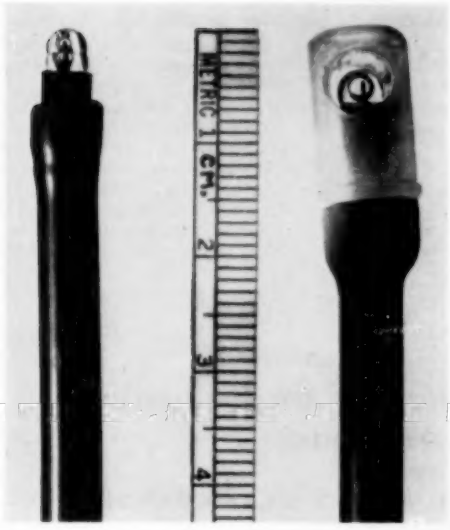


FIGURE 1.

Figure 3.

FIG. 2.—Proper position for stomach electrode.

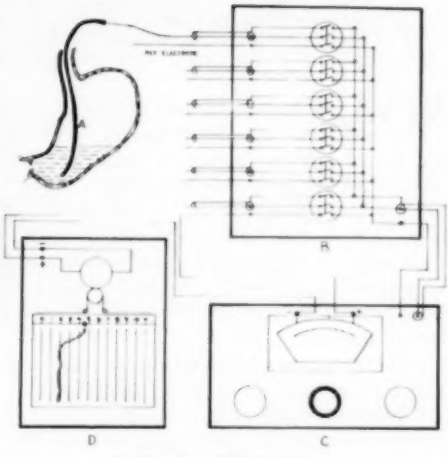


Fig. 4.—Diagram of instrumentation for recording gastric pH.
A = glass electrode in stomach
B = 6-Way manual switch
C = Beckman H-2 pH meter
D = Fisher Recordall Unit

METHODS

Instrumentation.—The instrumentation (Fig. 1) employed in the continuous recording of gastric pH was composed essentially of five integral components. These components are as follows: (a) Beckman stomach electrode, X800-22; (b) Beckman reference electrode 270; (c) Beckman H-2 pH meter; (d) Beckman six-point manual switch, 8700; (e) Fisher Recordall Unit. Each of these instruments is now commercially available.

The shielded lead wires of the stomach electrodes were passed through a 14 French Modified Levine tube in such a manner that the bulk of the electrode was left exposed. A small polyethylene guard was fashioned to fit over the electrode bulk leaving it exposed in its central lumen (Fig. 2). With an arrangement such as this, it was possible to keep the electrode from making contact with the gastric mucosa without preventing the intimate contact of the electrode with gastric secretions. This small "shielded" stomach electrode[1] was passed into the

[1] The tiny glass electrode suitable for use in the stomach was developed by the Beckman Scientific Instrument Company upon our urging that an electrode which could be depended upon to operate in any position would be far superior to those electrodes which had been previously available.

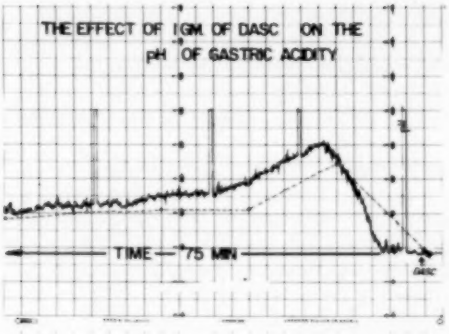


Fig. 5.—Broken line = intermittent sampling; Continuous line = original "electrometric recording."

stomach via the mouth and esophagus of each subject with a minimum of discomfort. Fluoroscopic viewing of each subject insured the proper positioning of the electrode within the stomach (Fig. 2). The "reference electrode" was placed in the circuit by applying it to the inner surface of the forearm. A gauze square saturated with saline was placed over the electrode and then this portion of the arm wrapped with plastic sheeting.

The electrodes, both the glass and the reference, were coupled with a manual six point switch box (Figs. 3, 4). The switch arrangement was such that six electrode assemblies could be coupled to it, however, at any one time only one electrode circuit could be activated for recording. It was possible to record the gastric pH of as many as five subjects intermittently at one time while the sixth electrode maintained in a buffer solution of pH 6 was used for standardizing and determining the extent of "drift" inherent to the recording apparatus.

The output of the switch box was fed into the pH meter through the regular electrode input assembly. The signal from the meter indicator was taken off the posts of the pH meter via a Fisher Recordall Accessory, from whence the signal was fed directly into the Recordall Unit. Output of the meter was recorded with a full scale deflection (of the recorder) being 1.1 volts. Therefore 100 mv. was equivalent to 1 inch of chart deflection. The pH, therefore, was recorded on the basis that one pH unit was equal to one inch of chart deflection. The Recordall Unit was equipped with a zero shifter so that any range of pH would be studied with an accuracy of 0.05 pH units. All electrode leads were 20 feet in length, thereby making it possible to keep the recording setup in a central location relative to the subjects. Shift in the recording apparatus was checked and corrected throughout the recording sessions by means of the standardizing electrodes which were maintained in a pH 6 buffer media. The apparatus showed some drift during the first half hour of recording, but thereafter drift was negligible.

Intermittent Gastric Aspiration.—Previously fasted, healthy, adult males, selected because they secreted large volumes of gastric juice with a high concentration of acid, were intubated via the nose with a standard Levine tube. The tube was passed through the esophagus into the stomach. An initial gastric sample (3 ml.) was withdrawn with the aid of a special glass syringe. Measurement of pH was made upon this sample, and only subjects whose gastric pH was 3.0 or below were selected for the study.

Administration of Antacids.—Antacids to be studied were administered as 1-Gm. doses in aqueous suspension or solution via the intubation tube. The antacid suspension which was maintained at 5 ml. was administered immediately after the initial gastric sample had been obtained.

Gastric Sampling.—Immediately after the zero or initial gastric sampling the antacid was administered as mentioned above. Every fifteen minutes thereafter, for a seventy-five minute period, 3-ml. samples of gastric juice were aspirated via the intubation tube. Each sample of gastric juice was examined for free and total titratable acidity employing Topfer's reagent and phenolphthalein as indicators. The determination of pH was made with a Beckman pH meter

Continuous pH Recording.—The "stomach electrode" was introduced into the mouth and swallowed by the subjects. Fluoroscopic scanning of the upper abdominal region determined the position and location of the electrode (Fig. 4). Continuous pH recordings were made over a seventy-five-minute observation period.

After the determination of the initial pH the antacids were suspended or dissolved in 30 ml. of tap water, and swallowed via the mouth and esophagus. It was necessary to employ this volume of sample since it was impractical to administer the antacids via the intubation tube.

RESULTS

Essentially, the data obtained by the continuous recording of pH were similar to those found after aspiration of gastric contents.

Within fifteen minutes 1.0 Gm. DASC produced a rise to approximately pH 5 which gradually decreased to pH 3.5 and remained there throughout the observation period (Fig. 5).

Within twelve minutes 1.0 Gm. aluminum hydroxide caused a rise in pH to approximately pH 6 which decreased rapidly to a level below pH 3 and remained there throughout the observation period (Fig. 6).

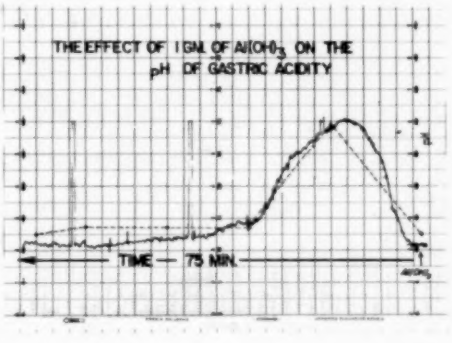


Fig. 6.—Broken line = intermittent sampling; Continuous line = original "electrometric recording."

Within fifteen minutes after the administration of 1.0 Gm. calcium carbonate a rise in pH to approximately pH 7 was observed, which remained above pH 6 over a forty-five-minute period when it dropped sharply below pH 3, and remained there for the remainder of the observation period (Fig. 7).

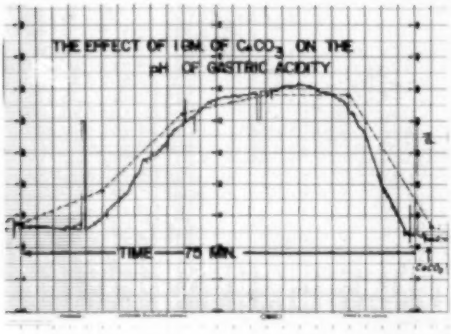


Fig. 7.—Broken line = intermittent sampling; Continuous line = original "electrometric recording."

Sodium bicarbonate, 1.0 Gm., within ten minutes, produced a rise in pH to approximately pH 7.5 which dropped slowly below pH 3 (possibly acid rebound) and remained there throughout the observation period (Fig. 8).

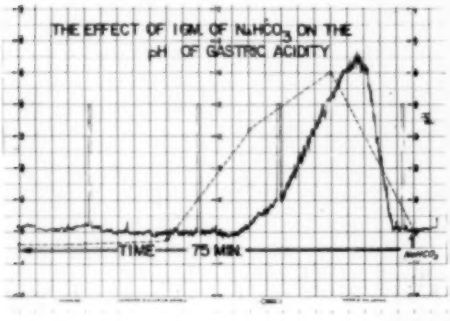


Fig. 8.—Broken line = intermittent sampling; Continuous line = original "electrometric recording."

Figures 5 to 8 are original tracings of the continuous recordings with the data obtained by sampling of gastric juice imposed thereon. The figures should be read from right to left. Each principal horizontal line represents one pH unit, each principal verticle line three minutes of elapsed time.

DISCUSSION

In the past, the effect of antacids upon gastric acidity have been investigated by intermittent aspiration of the gastric contents. There are, however, distinct disadvantages to this method. The acidity of the gastric contents determined electrometrically from the electrode in the stomach is far superior to the aspiration method since, no gastric juice, antacid, or acid is removed from the stomach.

It also enables the investigator to study the action of a particular agent over a long period of time without the chance of overlooking some important effects which could be missed between sampling, necessitated by the aspiration methods.

Although several instruments have been described for the continuous measurement of pH *in vivo*, the equipment which we have assembled, for the electrometric recording of pH within the stomach, has the following advantages. (a) The special stomach electrode records in a normal as well as inverted position. (b) The multiple switch arrangement allows studying many patients simultaneously. (c) The addition of the "reference electrode" reduces the possibility of inherent drift within the apparatus. (d) The polyethylene shield of our design prevents contact between the electrode and gastric mucosa.

This present study indicates clearly that the apparatus which we have assembled for measuring pH continuously is applicable for evaluating the *in vivo* effects of potential antacid agents. The results obtained electrometrically check so closely with the results obtained with aspiration techniques that we may be assured of the reliability of the data.

By comparative techniques, four antacid agents have been evaluated, for their effects upon the acidity of the gastric contents. As a criterion of effectiveness the pH range of 3 to 5.5 was chosen since in addition to neutralization, there is assumed to be little or no alteration in the normal physiologic processes of digestion within the stomach within this pH range.

Based upon the data accumulated by both the aspiration technique and the continuous pH recording method, the antacids in decreasing order of their effectiveness were dihydroxy aluminum sodium carbonate, aluminum hydroxide, calcium carbonate, and sodium bicarbonate.

REFERENCES

(1) Flexner, J., Kniazak, M., and Nyboer, J., *Science*, **80**, 239(1939).
(2) Flexner, J., and Kniazak, M., *Am. J. Digest Diseases*, **7**, 138(1940).
(3) Rosset, N. E., and Flexner, J., *Ann. Internal Med.*, **18**, 193(1943).
(4) Rovelstad, R. A., *Gastroenterology*, **31**, 530(1956).
(5) Rovelstad, R. A., Owen, C. A., Jr., and Magath, T. B., *ibid.*, **20**, 609(1952).
(6) THIS JOURNAL, **46**, 545(1957).

A New Accurate and Reliable Method to Record Blood Coagulation Time Using an A. C. Bridge Principle*

By ALFRED W. RICHARDSON† and JACK G. BISHOP‡

There has been a continuous effort among workers in the field of blood coagulation to design or modify a method of measurement that might possess greater reliability than existing methods. The method described here, which offers a clotting time of about six minutes when compared to existing methods, is considerably more reliable; having been demonstrated to evidence a standard error of ±18 seconds, and permitting a minimum of subjective participation error in the measurement process. This device does not, however, eliminate the inherent contribution of the method of blood withdrawal; therefore a rigidly standardized withdrawal procedure is recommended.

DURING THE PAST fifty years numerous methods have been devised to measure the coagulation time of blood by detecting changes in conductivity during the process of coagulation. Early methods such as that of Frank (1) lacked sufficient sensitivity to detect any significant alteration of electrical conductivity in whole blood during the clotting process. Rosenthal and Tobias (2), by the use of a more sensitive impedance bridge arrangement, were able to measure small increments of impedance alteration in whole blood during the period of clot formation. Later Henstell (3) confirmed this finding by the use of a similar method of measurement, but employing a different frequency of oscillation of the impedance bridge. These methods share the common limitation that the measurement is affected by the blood in both the liquid and the gel state. The electrical characteristics of these two states are quite close, with a given volume and geometric configuration. This small difference, in ratio to the total electrical characteristic, allows measurable intervening variables. Hirsch and his colleagues (4) used a similar measuring apparatus to determine the hematocrit of blood.

Problems with the use of electrical techniques have led to the widespread utilization of mechanical methods for the measurement of clotting time such as that of Lee and White (5), and modifications like that of Macht and Hoffmaster (6). Considerable knowledge has been learned about the mechanism of blood coagulation by the employment of newer mechanical and electrical methods. Macht and Hoffmaster demonstrated that blood coagulation does not occur as a sudden discrete event, and Sigman, *et al.* (7), have shown variation in the agitation of blood to be a factor in measurement error. From this evidence one might conclude that any motion of the blood should be uniform, if a maximal reliability of the measurement is to be achieved.

With these findings in mind, a conductivity method to measure the coagulation time of blood was devised by Richardson and Bishop (8) in 1953 which intermittently assayed the direct current conductivity of the isolated clot at 10-second intervals. This method reproduced the photometric clotting curve of the coagelgram of Nygaard (9), but in mirror image due to the different inherent physical characteristics of the two methods. Later Bishop and Richardson (10) modified the direct current conductivity method so as to measure the activity of proteolytic enzymes.

METHOD

Described here is a modification of the original method of Richardson and Bishop (8) which features the same motor-driven oscillating platform and circuit-switching arrangement, but which employs a polystyrene blood container and alternating-current bridge circuit with a dual triode amplifier circuit. By the switching arrangement, only the formed clot, and not the whole blood, is assayed. As the clot is formed, the a.-c. bridge is progressively unbalanced, thus increasing the alternating forcing potential on the first grid. It is amplified and rectified to be recorded on 1-ma. or 5-ma. recorder, the recorded ordinate being directly proportional to the height of the forcing potential. This method differs from that of Rosenthal and Tobias (2) and Henstell (3) in that the clot itself is selectively assayed. It reproduces the clotting curve reported by Nygaard (9) and by Richardson and Bishop (8) with linear amplification. This new method has been in use more than a year.

Figure 1 is a diagram of the circuit employed.

* Received March 13, 1957, from St. Louis University School of Medicine, St. Louis, Mo.
This work was supported by grants from Flint, Eaton and Company, Decatur, Ill.
† Department of Physiology, St. Louis University School of Medicine.
‡ Department of Physiology, Baylor University School of Dentistry, Dallas, Texas.

The basic balanced bridge uses four 3K-ohm precision resistors, with a 25K-ohm balance control and a 10K-ohm phase control, arranged in such a manner that a 6.3-volt a.-c. source from the filament circuit connects to positions 1 and 4, and events in the blood-container circuit affect the resistance between positions 3 and 4 by a parallel connection. The formation of a clot in the blood container progressively lowers this resistance and allows an alternating potential to be passed to the dual triode

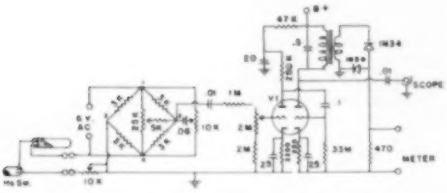


Fig. 1.—A schematic diagram of the circuit of the blood coagulation meter showing: the input arrangement; the 60-c.p.s. a.-c. bridge; dual-triode, amplifying circuit; and output, which is rectified for the use of a d.-c. meter.

amplifier V, the gain being adjustable by a 2-megohm gain control. Any medium mμ triode may be used for V, such as 6SN7, 6S78, or 12AU7 (6SN7 used here). An audio output transformer is used in the plate circuit of the second stage, the output being rectified by two 1N34 diodes. The 470-ohm resistor in the output acts as a meter shunt, so that a 1500-ohm 1-ma. or a 70-ohm 5-ma. ink-writing recorder can be used interchangeably.

A B-voltage supply of 150 to 300 volts is satisfactory for this apparatus, and is usually available, or can be built from commercial kits. Figure 2

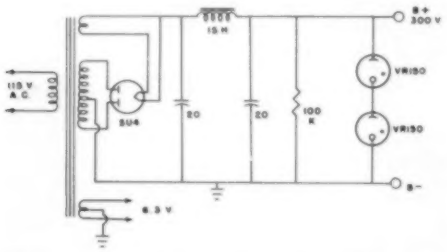


Fig. 2.—The schematic diagram of a simple, very adequate power supply. It can be used with or without voltage regulation, such regulation being suggested only when there are power-line fluctuations of consequence.

shows a simple and very satisfactory power-supply circuit that can be constructed. It may be used with or without the two voltage regulating tubes, VR150.

The use of an oscilloscope connected through a condenser to the second plate circuit is not necessary for the operation of the instrument, but is very useful initially as a monitor when the controls are set at a null balance for the bridge. It is important to adjust the phase control so that the potentials at connections 2–3 and 1–4 appear 180° out of phase, shown as a straight line on the oscilloscope when the balance control is moved to a null position.

The complete apparatus includes a 115-v. a.-c. motor attached to a gear that oscillates back and forth through 90° in such a manner that an attached platform will swing up and down from horizontal. A full oscillation occurs every ten seconds. The motor and gear assembly can be purchased commercially, as a unit. Blood is placed in a container on the platform along with a mercury switch arranged with the switch and probes in reverse positions as shown in Fig. 1. With this arrangement, when the blood is liquid it rolls away from the probes before the series switch closes, and therefore does not unbalance the bridge. When, however, clotting begins, the clot cannot flow away so that there is conductivity when the switch closes, the bridge is unbalanced in ratio to the magnitude of the clot, and the events are recorded on the output recorder.

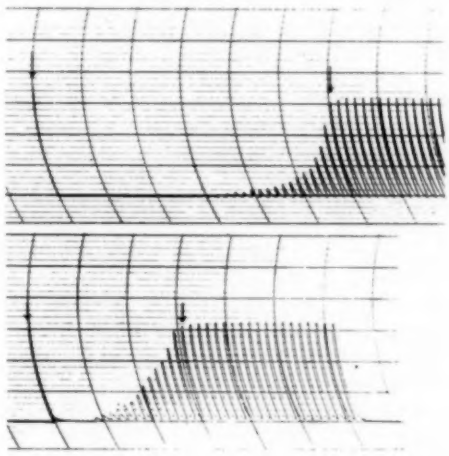


Fig. 3.—Two sample records of the progressive events in blood coagulation. Arrows indicate the points of withdrawal of blood and the point in time on each record where a firm, visible clot was formed. The top record demonstrates a clotting time of 6.0 minutes, the lower record a clotting time of 3.1 minutes. It is apparent that there is no difficulty in the determination of different blood clotting times, or the periods involved in the slow and accelerated phases of clotting.

Figure 3 is a photograph of two clotting records of the blood of dogs, using 1 ml. of blood in polystyrene tubes 1.0 × 5.0 cm. These records were chosen from a large series to show the difference in appearance of two different clotting time durations. The absolute height on the ordinates is adjustable and not significant, but the relative height on any curve during the coagulation at each point in time is significant, since it represents the progress of the clotting events. A clear delineation of the slow phase of clotting and the accelerated phase is apparent in both records, suggesting that this method

might well be employed in detailed studies of the specific factors involved in the clotting mechanism.

The use of a balancing bridge is of considerable advantage in detailed studies of clot formation. By using a negative bridge balance, any conductive effects of probe wetting may be balanced out of the recording, leaving only a record of clot formation. Adjustment of the 10K-ohm control (Fig. 1) in series with the probe circuit at the input allows a linear response to accumulating blood-clot volume when the bridge is being unbalanced. Studies conducted in the laboratory have shown this instrument to be considerably accurate and reliable in blood coagulation assays. There is an inherent error of five seconds in the method due to electrical sampling procedure, and a standard error of the mean was determined as eighteen seconds in a series of control assays where the average clotting time was 6.4 minutes. The major variables in clotting time have been found to be in the method of blood withdrawal, and in the choice of surface material to which the blood is exposed in the blood receptacle. The assays reported above were with the use of polystyrene receptacles, and platinum probes housed in a plastic-coated stopper for the electrode assembly.

RESULTS

Table I summarizes the results of seven series of assays. Two types of control conditions were analyzed which demonstrate the effect of the technique of blood withdrawal utilizing 5-cc. glass syringes and No. 21 hypodermic needles. When the syringes and needles were freshly flushed with paraffin oil, the average clotting time for blood was 6.4 minutes, as compared with five-and-a-half-minutes clotting time when they were filled with paraffin oil, flushed out, and allowed to drain for thirty minutes. This difference was significant at the 1% level of confidence. Withdrawn blood remained in the syringes thirty seconds before being transferred to the polystyrene receptacles used throughout the assays.

Also included in Table I are five groups of controlled experimental assays using small concentrations (20–500 p. p. m.) of agents *in vitro* known to diminish blood-clotting time. These assays demonstrate the ability of the coagulation meter to quantitate coagulant actions on blood. All agents at these concentrations accelerated clotting time, significantly different from the controls at a 1% level of confidence, except added calcium ion, which evidenced a difference at the 2% level of confidence.

Other experimental assays were conducted to study anticoagulant actions. These included larger concentrations of EDTA and Ceanothyn (both of which remove calcium and therefore act diphasically) and heparin. Plots of the results of these studies showed anticoagulant actions as a function of increased concentration as would be anticipated by use of any other accurate method of blood coagulation measurement.

TABLE I.—RESULTS OF QUANTITATIVE ASSAYS USING AGENTS KNOWN TO AFFECT BLOOD COAGULATION

Agent	Concn. in Blood, ppm.	N	Minutes Clotting Time		Difference[a] Min.	Difference[a] %
Calcium chloride (value as calcium)	20[b]	18	4.3	...	−0.7	−14
Controls	...	24		5.0	...	..
Thromboplastin (commercial)	500	60	3.5[d]		−2.9	−45
Controls	...	58	...	6.4[d]	...	..
Thrombin (commercial)	100	14	3.6[d]	...	−2.6	−42
Controls	...	18	...	6.2[d]	...	..
Ethylene-diamine tetraacetic acid[c]	38	27	3.4	...	−2.1	−38
Controls	...	36	...	5.5	...	..
Ceanothyn	200	36	3.9	...	−1.7	−30
Controls	...	42	...	5.6	...	..
Control Results of Two Blood Withdrawal Techniques						
Syringes flushed with paraffin oil						
A. Freshly flushed		382		6.4	...	..
B. Drained for 30 minutes		422		5.5	−0.9	−14

[a] 1% level of confidence, or better, except calcium assays (2%).
[b] By weight, others by volume.
[c] Standard solution: 32 mg. Ca chelated by 1 ml. of EDTA solution.
[d] Syringes freshly flushed with paraffin oil.

REFERENCES

(1) Frank, R. T., *et al.*, *Blood*, **5,** 1017(1950).
(2) Rosenthal, R. L., and Tobias, C. W., *J. Lab. Clin. Med.*, **33,** 1110(1948).
(3) Henstell, H. H., *Am. J. Physiol.*, **158,** 367(1949).
(4) Hirsch, F. G., *et al.*, *Blood*, **5,** 1917(1950).
(5) Lee, R. I., and White, P. D., *Am. J. Med. Sci.*, **145,** 495(1913).
(6) Macht, D. I., and Hoffmaster, T., *Science*, **115,** 91 (1952).
(7) Sigman, E., *et al.*, *Am. J. Physiol.*, **118,** 708(1937).
(8) Richardson, A. W., and Bishop, J. G., *Science*, **117,** 37(1953).
(9) Nygaard, K. K., "Hemorrhagic Diseases," C. V. Mosby Co., St. Louis, Mo., (1942).
(10) Bishop, J. G., and Richardson, A. W., *J. Lab. Clin. Med.*, **43,** 327(1954).

An Investigation of the Possible Potentiation of the Analgetic Properties of Morphine by Mephenesin*

By SUMNER M. ROBINSON† and HOWARD J. JENKINS

Results of an attempt to determine the existence of potentiation of morphine analgesia by mephenesin are presented. Data collected in a quantal application of the Hardy-Wolff-Goodell Dolorimeter with rats as test animals provide evidence of the extent of this potentiation.

MEPHENESIN, one of a series of α-substituted glycerol ethers, has been found to produce in moderate doses in animals a profound muscular relaxation and, in higher doses, analgesia, ataxia, arouseable sleep, and complete paralysis (1–3). The action of mephenesin is postulated as a differential depression of function in the basal ganglia, brain stem, thalamus and, in larger doses, the internuncial neurons (4). Its combination with codeine, in Tolserol® with codeine, suggested a study of possible potentiation of morphine by mephenesin.

Literature pertaining to the action of morphine and mephenesin in combination has been scanty and contradictory. Levi (5) stated that hypnotic synergism was observed when mephenesin and various narcotics were given simultaneously by intravenous administration to dogs and rabbits. Among the agents were morphine, chloroform, ether, urethane, and several thiobarbiturates. Lang, *et al.* (6), showed that mephenesin, given orally to mice at 300 mg./Kg., exhibited no signs of synergism with ether, chloroform, or morphine.

In contrast, it is interesting to note the agreement of the investigators on the synergism of mephenesin and the barbiturates. Berger and Bradley (2) found that doses of mephenesin and hexobarbital, which alone were ineffective when injected together in rabbits, produced deep narcosis. Levi (5) observed that sleeping time in dogs and rabbits was substantially increased when Pentothal sodium and mephenesin were administered in combination. Five milligrams/kilogram of Pentothal alone failed to produce anesthesia, while intravenous administration of this dose and 15 mg./Kg. of mephenesin together produced anesthesia of six minutes duration in 90% of the aniamals, an effect produced only with 7 mg./Kg. of Pentothal alone. Lang (6) noted that only five per cent of the mice, given 50 mg./Kg. of Pentothal and 50 mg./Kg. of mephenesin I. P. in combination, had recovered their righting reflexes after five and one-half hours, while 50 per cent of the mice given Pentothal alone had recovered.

EXPERIMENTAL

To detect any potentiation, a quantal application of the Hardy-Wolff-Goodell Dolorimeter was employed (7, 8). The response of like groups of Carworth-Wistar rats to different dosage levels of morphine alone and in combination with or prior to mephenesin was determined on the basis of tail flick to constant radiant heat (stimulus) intensities; applied both before and after the drug(s) for constant exposure periods. For evaluation of the data, the Litchfield-Wilcoxon method was used (9). The analgetic effectiveness of morphine, expressed as an AD_{50} with confidence limits for 19/20 probability, was compared with the analgetic effectiveness of morphine administered in combination with and at various intervals prior to mephenesin, similarly computed and expressed.

Sixty male rats of the Carworth-Wistar strain were divided into three groups of 20 rats each. Each group of 20 animals, then, represented a dosage level. The test animals varied in weight from 80 to 100 Gm. at the beginning to 200–250 Gm. at completion of the study. All the animals were maintained on Rockland diet and water, *ad lib.*, and kept in a temperature-controlled room at 70° F. ± 2°.

The solution of morphine sulfate was so prepared, when given alone, that the calculated dose per 100 Gm. of body weight was contained in 0.5 ml. When given in combination with or prior to mephenesin, the solution contained the calculated dose in 0.25 ml. Dilutions of mephenesin were prepared from Tolserol solution, a 2% solution of mephenesin in isotonic sodium chloride. The mephenesin solution also contained the calculated dose per 100 Gm. of body weight in 0.25 ml. All injections were made subcutaneously, under the skin of the back.

Prior to application of the radiant heat stimuli, the tips of the tails were blackened with lampblack (10). Each rat, gently restrained by hand, was subjected to a response check with the same series of heat stimuli as was later used in the testing. In all the testing the exposure period of the stimulus was kept constant at three seconds, while the interval between successive stimuli was thirty seconds in every instance. The first stimulus of 450 mc. (millicalories) was followed after thirty seconds by a stimulus of 320 mc. and, if no tail flick was obtained, a second stimulus of 320 mc. thirty seconds later. Following administration of the drug(s), failure to

* Presented to the Scientific Section, A.PH.A., Detroit meeting, April, 1956.
From a thesis submitted to the Massachusetts College of Pharmacy by Sumner M. Robinson in partial fulfillment of the requirements for the degree of Master of Science.
† Present address: Department of Biological Science, Massachusetts College of Pharmacy, Boston.

respond to either the first or second stimulus of 320 mc., whichever was previously effective, evidenced the designated analgesia.

For determination of the AD_{50} of morphine alone, dosage levels of 1 mg./Kg., 3 mg./Kg. and 5 mg./Kg. were selected. Twenty rats were tested in each dosage level, so that determination of the AD_{50} depended upon the response of 60 animals. The procedure for an individual dosage level was as follows: after a response check of the dosage group of 20 rats, injections at the selected level were made at intervals of two minutes. Immediately following the last injection, the rats were tested for evidence of analgesia in the same order as injected, two minutes being allowed for checking each animal. The forty-minute interval between injection and response check has been indicated to be adequate for the peak analgetic effect of morphine (10). This procedure was then followed with each of the two remaining groups of 20 rats, each group receiving a different dose of the drug.

In testing for potentiation, it was decided to administer morphine twenty minutes prior to mephenesin and, in later studies, in combination with mephenesin. The selection of a twenty-minute interval between the administration of morphine (whose peak effect is achieved in approximately forty minutes) and the administration of mephenesin would allow this testing to be completed in approximately the same period of time as required for the testing of morphine alone and the testing of morphine in combination with mephenesin, provided that the mephenesin and morphine injections were each made at one-minute intervals in each segregated dosage level. During the potentiation studies, the dosage levels of morphine were reduced to 0.5 mg./Kg., 2 mg./Kg. and 3.5 mg./Kg.

RESULTS AND DISCUSSION

Table I shows the AD_{50}'s of morphine alone, Table II the AD_{50}'s of morphine administered twenty minutes prior to mephenesin, and Table III the AD_{50}'s of morphine administered in combination with mephenesin. Ten milligrams/kilogram of mephenesin injected twenty minutes after morphine resulted in a series of morphine AD_{50}'s ranging from 1.0 mg./Kg. to 1.37 mg./Kg. These values fail to come within the 19/20 confidence limits of the standard (original) AD_{50} of morphine alone (1.59 mg./Kg. to 2.77 mg./Kg.). This is evidence of the extent of the potentiation of morphine by mephenesin when the mephenesin is administered shortly after morphine.

The same dose of mephenesin injected in combination with morphine resulted in a series of morphine AD_{50}'s ranging from 1.05 mg./Kg. to 1.3 mg./Kg. These also fail to come within the confidence limits of the standard AD_{50} of morphine. This is evidence of the extent of the potentiation of morphine by mephenesin when mephenesin and morphine are administered together.

The data of the two procedures indicate that there is no significant difference in the ability of mephenesin to potentiate morphine when the former is administered in combination with or shortly after the latter.

During this work the question arose as to whether these combination and split-time values are a true indication of potentiation of analgesia. The tail flick, as elicited by the Dolorimeter, has been described by Irwin, *et al.* (11), as a spinal reflex, while Bonnycastle and Mollard (12) are of the opinion that rats must be trained to withdraw the tail from the radiant heat stimulus. A component of the action of mephenesin is said to involve a depression of spinal reflexes (13). It follows, therefore, that if tail flick is assignable to these reflexes, mephenesin could possibly give false evidence of analgetic potentiation when administered with morphine. With a control group of twenty animals, it was found that

TABLE I.—MORPHINE AD_{50} DETERMINATIONS

Morphine Dosage (mg./Kg.)	Number Analgesia	Analgesia, %	Morphine AD_{50} (mg./Kg.)
1	4/20	20	
3	12/20	60	
5	19/20	95	2.1 (1.59–2.77)
1	5/20	25	
3	12/20	60	
5	19/20	95	2.1
1	3/20	15	
3	9/20	45	
5	16/20	80	2.8
1	4/20	20	
3	12/20	60	
5	17/20	85	2.23

TABLE II.—MORPHINE AD_{50} DETERMINATIONS[a]

Morphine Dosage (mg./Kg.)	Number Analgesia	Analgesia, %	Morphine AD_{50} (mg./Kg.)
0.5	5/20	25	
2.0	11/20	55	
3.5	18/20	90	1.24
0.5	4/20	20	
2.0	12/20	60	
3.5	19/20	95	1.14
0.5	5/20	25	
2.0	10/20	50	
3.5	18/20	90	1.37
0.5	6/20	30	
2.0	13/20	65	
3.5	18/20	90	1.0
0.5	13/20	15	
2.0	12/20	60	
3.5	17/20	85	1.37

[a] With mephenesin in split-time administration.

TABLE III.—MORPHINE AD_{50} DETERMINATIONS[a]

Morphine Dosage (mg./Kg.)	Number Analgesia	Analgesia, %	Morphine AD_{50} (mg./Kg.)
0.5	7/20	35	
2.0	11/20	55	
3.5	17/20	85	1.2
0.5	4/20	20	
2.0	12/20	60	
3.5	17/20	85	1.3
0.5	6/20	30	
2.0	13/20	65	
3.5	18/20	90	1.05

[a] With mephenesin in combination administration.

mephenesin alone, in doses as high as 50 mg./Kg., did not alter the pre-test response check pattern either twenty minutes or forty minutes after injection. This dose is five times the dose of mephenesin used in testing for potentiation. This indicates that either the tail flick is not a spinal reflex or that, as a spinal reflex, it is not affected by any but higher doses of mephenesin. On the other hand, since morphine exerts a stimulant action on the cord, it is not likely that it potentiates the depressant action of mephenesin on the spinal cord.

That the variation in the weight of the animals had no effect on analgetic response is seen in a comparison of the original AD_{50} (and confidence limits) of morphine alone with the AD_{50}'s of morphine alone determined at various intervals throughout the investigation. Thus, 2.1 mg./Kg. represents the AD_{50} of morphine alone when the rats weighed least while 2.23 mg./Kg. represents the AD_{50} of morphine at the completion of this investigation, when the animals weighed most. This would seem to indicate that variation in weight had little effect upon analgetic response, and that tolerance did not occur during the course of the investigation.

SUMMARY

1. A quantal application of the Hardy-Wolff-Goodell Dolorimeter is employed for the investigation of the possible potentiation of the analgetic activity of morphine by mephenesin. Rats are used as test animals, and the experimental data evaluated by the Litchfield-Wilcoxon method.

2. Evidence of the extent of potentiation is provided when the analgetic dose 50% (AD_{50}) of morphine alone is compared with the analgetic dose 50% of morphine injected in combination with and prior to mephenesin.

3. The degree of potentiation in both the combination and split-time studies appears to be of the same magnitude.

REFERENCES

(1) Berger, F. M., and Bradley, W., *Brit. J. Pharmacol.*, **1,** 265(1946).
(2) Berger, F. M., and Bradley, W., *Lancet*, **252,** 97 (1947).
(3) Berger, F. M., and Bradley, W., *Brit. J. Pharmacol.*, **2,** 241(1947).
(4) Gammon, G. D., and Churchill, J. A., *Am. J. Med. Sci.*, **217,** 143(1949).
(5) Levi, A., *Boll. soc. med.-chir. Modena*, **48,** 529(1948).
(6) Lang, D. A., Kimura, K. K., and Unna, K. R., *Arch. intern. pharmacodynamie*, **85,** 257(1951).
(7) Hardy, J. D., Wolff, W. G., and Goodell, H. J., *J. Clin. Invest.*, **19,** 649(1940).
(8) Christensen, B. V., and Tye, A., THIS JOURNAL, **40,** 404(1951).
(9) Litchfield, J. T., and Wilcoxon, F., *J. Pharmacol. Expil. Therap.*, **96,** 99(1949).
(10) Lindsay, R. T., Master of Science Thesis, Massachusetts College of Pharmacy, 1955.
(11) Irwin, S., Houde, R. W., Bennett, D. R., Nendershot, L. C., and Seevers, M. H., *J. Pharmacol. Expil. Therap.*, **101,** 132(1951).
(12) Bonnycastle, D. D., and Mollard, J., *Federation Proc.*, **7,** 206(1948).
(13) Henneman, E., Kaplan, A., and Unna, K., *J. Pharmacol. Expil. Therap.*, **97,** 331(1949).

A Method for the Measurement of Lubricating Property of Medicinal Products*

By GERHARD LEVY and T. W. SCHWARZ

The need for the evaluation of lubricants used in medical practice disclosed a lack of any instrument or quantitative method for the determination of lubricating property, or lubricity. The conditions required for testing medicinal lubricants are compared with those used in the testing of industrial lubricants. An instrument is described which is designed to measure the lubricant effect of medicinal products under conditions approaching normal use. The range and the accuracy of the instrument are illustrated by use with several classes of lubricants.

THE RESISTANCE resulting from relative motion of two bodies in contact with each other is termed friction. In medical practice, friction is encountered in the course of numerous procedures such as by the insertion of catheters, sounds, and specula into anatomical passages, or by massages. To overcome the effects of friction, lubricants are used. To a large extent, lubrication applies to friction between solid boundaries. Unlike hard, rigid surfaces, which are typical in engineering practice, friction surfaces in medical practice are of various nature: liquid, semiliquid (mucous), nonrigid (skin), rubber of gloves and catheters, and the hard metallic surface of instruments. In a mechanical sense, friction consumes energy and generates heat. The effect of friction found in clinical practice is reflected by pain,

* Received February 28, 1957, from the University of California School of Pharmacy, San Francisco.
This study was conducted in part under a contract with the Armed Services Medical Procurement Agency.
The authors wish to acknowledge the cooperation of James L. Plumb, Senior Instrument Mechanician, in the construction of the lubrimeter.

mucous membrane or skin injury, irritation, difficulty in instrumentation, and the resultant decrease in patient cooperation.

The reduction of friction by lubrication is accomplished by a variety of substances and the relative evaluation of lubricants has become a specialty of complex instrumentation. Products such as jellies, fixed oils, emulsions, and polyhydroxy compounds (glycerin, propylene glycol) are used as lubricants in medical practice. Their evaluation has not been attempted with anything that is even remotely comparable to the exactitude of engineering methods. Understandably, the behavior of two solid, easily reproducible surfaces in relative motion to each other is more easily observed and measured under controlled conditions than the motion of two surfaces, one of which is mucous in character, nonrigid, and hardly reproducible. A method that aims to compare lubricants used in pharmaceutical practice can, therefore, at best, only approach conditions of actual use. In engineering practice, lubricants can be evaluated under well-defined conditions, such as operating speed and dimensions of the system to be lubricated, temperature and pressure, and character of surfaces. Thus the degree of surface wear, the amount of friction loss, the amount of heat generated, mechanical efficiency, load-carrying capacity—any or all of these contribute to defining the property of a lubricant in engineering.

On the other hand, the evaluation of lubricants for skin and mucous surfaces cannot be carried out by the same methods. These structures differ in shape, in the amount and consistency of mucus or sebum present, and in the condition of epithelial surfaces, among others. There are also constant changes in clearance, as for instance, between the walls of the urethra and a catheter inserted into it. Mucous membranes are covered with a fairly cohesive, aqueous film. This is adequate for normal physiological function. Upon passage of an instrument, however, the natural thin film proves to be inadequate. Additional lubricant has to be introduced. This condition with ample lubricant present is, in engineering terminology, called "full fluid lubrication." The instrument described here was designed to compare lubricants under this condition of "full fluid lubrication."

In our search for a method of comparing lubricants, we turned first to the instruments used by lubricating engineers. Various such testing devices were considered (1–3). High pressure, high operating speeds, and considerable heat generation make these instruments inapplicable to surfaces of physiological nature or to the testing of aqueous lubricants. Nor can methods that measure the wear of contacting surfaces, the amount of heat generated, or the load which causes sufficient friction to stop motion ("load-carrying capacity") be applied to skin or mucous membranes or materials resembling them. Furthermore, the composition of most lubricants for medicinal use is fundamentally different from those used in engines; they would not stand up if subjected to testing procedures common for mechanical lubricants.

A search of the literature failed to disclose any instrument or quantitative method for the determination of lubricant property in medicinal products. An instrument intended to evaluate the lubricant effect of products used on the skin or mucous membranes must operate under conditions which resemble those ordinarily found in the clinical use of these products. The motion of the test surfaces relative to each other must be rather slow, the pressure between these surfaces must be low, and the character of the surfaces should in the ideal case resemble that of skin or mucous membranes. Under such conditions, there is no determinable generation of heat, and the applied load is hardly sufficient to stop the moving surfaces. There is no possibility of determining quantitatively the extent of surface wear, or damage due to the lack of severe operating conditions, and there remains the difficulty of relating damage of skin or mucous membranes to that of any other material which may be used as the test surface.

APPARATUS

Description of the Instrument.—The instrument, which the authors designed and called "Lubrimeter" may be operated at low speed and pressure. Its test surface is adaptable to different materials used in an attempt to approach physiological surfaces (Figs. 1 and 2).

The lubrimeter consists of a polished brass roller, which is rotated by a synchromotor at various selected constant speeds. On this roller rests a drum, larger in diameter than the brass roller, and made of Bakelite®. This Bakelite drum can be covered with cloth, rubber, skin section, or whatever material is to be used as the test surface. The drum is free to rotate around its own axis in either direction. It is hollow and has removable ends so that it may be weighted down by filling it with water, mercury, or other heavy material. In this manner the pressure between the surfaces of the roller and the drum may be conveniently altered. Underneath the roller is a pan that serves as a reservoir for the lubricant. The roller is partly immersed in the pan. As the roller turns, it picks up lubricant. In order to maintain a film of fairly uniform thickness on the roller surface, a retaining plate, whose edge is parallel

to the roller surface, scrapes off the excess of lubricant. The distance between the edge of the retaining plate and the roller surface is adjustable.

As the roller rotates at a slow, constant speed, the drum, which rests on the roller, is turning also—due to the friction. While the roller rotates clockwise, the drum turns in the opposite direction. The drum is connected to a spring by means of a cord which winds around a groove at one end of the drum. As the drum turns, the spring is stretched. The greater the frictional force, the longer is the extension of the spring; also greater is the tendency of the spring to contract (restoring force). A stop prevents the drum from turning more than half its circumference.

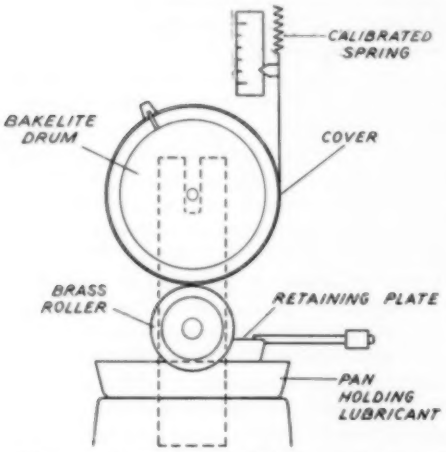


Fig. 1.—Diagram of Lubrimeter. Side view. Dimensions: Motor, G. E. synchronous inductor motor, torque 75 in./oz.; Diameter of brass roller, 3.62 cm.; Length of brass roller, 12 cm.; Diameter of drum, 7.5 cm.; Length of drum, 14 cm.

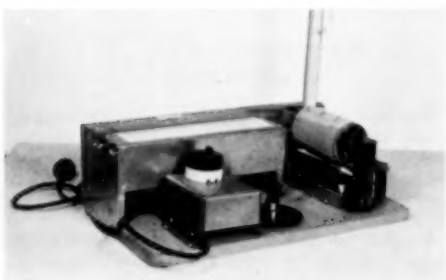


Fig. 2.—The lubrimeter.

A pointer is attached to the spring to simplify readings. The point of greatest extension of the spring is equivalent to the maximum friction exerted. The frictional force can be expressed in grams by appropriate calibration of the spring. By choosing a spring of proper stiffness, equilibrium between frictional force and restoring force occurs before the drum is turned to its extreme position (which is half the circumference from the rest position) and before the elasticity limit of the spring is reached.

Friction between roller and drum may be measured in the absence of a lubricant, and in the presence of various lubricants. The presence of a lubricant results in a decrease of friction, which is indicated by a lesser extension of the spring. The effect of lubrication may be expressed as friction values (in grams) with and without lubricant, or as the ratio of the two; in the latter case, the resulting fraction indicates the decrease in friction due to the lubricant. Or, where the chosen surface (skin, e. g.) is unsuitable for a "dry" run, the friction value of a test material may be compared to that of a reference lubricant.

While a variety of materials, such as rubber sheeting and skin, may be used as a cover for the upper roller, we found ribbed sheeting advantageous because excess lubricant will pass along the ridges rather than accumulate on one side of the two contacting rollers. Such accumulation produces a "drag," which may interfere with the accuracy of the measurement.

The springs were of copper-beryllium alloy, which has a high resistance to fatigue, and of stainless steel. Springs of varying stiffness were used to accommodate a wide range of frictional values.

The measurements reported here were made at an operating speed of 53.3 r. p. m. The weight of the drum was 550 Gm. It was covered with ribbed polyethylene sheeting.[1] The clearance between brass roller and retaining plate was 0.07 mm.

PROCEDURE

A polyethylene sheet is thoroughly cleaned with alcohol and air-dried. The sheet is then placed around the drum and fastened with pins. The roller is cleaned with acetone or ether and also air-dried. The pan is filled with the lubricant to be tested and placed underneath the roller so that the roller is partly immersed in lubricant. The roller is set in motion by the motor and the drum, which has been connected to the spring by a cord, is set on the roller.

As soon as the pointer attached to the spring reaches equilibrium, a reading is taken. It may require several minutes before equilibrium is reached. Under certain conditions, e. g., high friction and stiff spring, the spring may alternately expand and contract. In this case, the readings are made at maximum expansion. After a run has been completed, the polyethylene sheet is removed and replaced by another piece of polyethylene, and another reading taken. Since the surface of the polyethylene sheeting shows some slight variations, we have tested each lubricant with six sheets and taken the average of the readings.

RESULTS

The lubricant effect of five different preparations, representing different classes of medicinal lubricants, was determined with the lubrimeter. After the runs with six different sheets of polyethylene for

[1] Handi-mat, Palo Laboratories Supplies, New York.

each of the five products were completed, the lubrimeter and the sheets were thoroughly cleaned and the procedure repeated. The averages of the two runs differ by less than 10% (Table I).

TABLE I

Material	Classification	Av. of 6 Readings First Run, Gm.	Av. of 6 Readings Second Run, Gm.
Distilled water	Inorganic liquid	210	203
Tragacanth jelly 1.5%; 57,000 c. p. s.[a]	Aqueous jelly	173	174
Plastibase, hydrophilic[b]	Ointment	59	54
Glycerin 95%	Organic liquid	22.7	22
Liquid petrolatum	Oil	13.7	13.8

[a] Brookfield viscometer, model LVF, spindle No. 4, 6 r. p. m. at 25°.
[b] E. R. Squibb and Sons.

The value in grams represents the degree of friction. Liquid petrolatum produced the least friction, thus showing the best lubricity of the five materials tested.

In order to establish a base line, it was attempted to obtain a reading of friction in the absence of any lubricant. Due to the high degree of friction involved and the difficulty of obtaining truly "clean" surfaces, this value could not be accurately determined, but lies in the neighborhood of 440 Gm. for the polyethylene sheets used.

DISCUSSION

Accuracy.—The accuracy of the instrument depends greatly on the type of lubricant tested. Semisolids such as Plastibase spread less readily and are less readily picked up by the brass roller than liquids. Very good lubricants such as glycerin and liquid petrolatum reduce friction so much that measurements may be more difficult due to the lack of responsiveness of the instrument in this range even with a weak spring. To test the reproducibility of the data, the friction values of tragacanth jelly (1.5%, 57,000 c. p. s.) were determined in three runs, each run consisting of the determination on six different polyethylene sheets. A full tabulation is given in Table II. This table shows the variations from sheet to sheet and from measurement to measurement with the same sheet. The sheets were thoroughly cleaned between each series.

Significance.—The experimental results indicate that the lubricant effect of various classes of medicinal lubricants, organic and inorganic, liquid and semisolid, may be determined with the lubrimeter. The various materials exhibited greatly differing lubricant effects which show the necessity for an evaluation of this property whenever preparations are to be used as medicinal lubricants.

The relative friction values obtained on polyethylene surfaces were practically unchanged when polyethylene was replaced by ribbed rubber sheeting. These values also seem to agree with a perceptive evaluation on human skin, as far as such a totally subjective comparison is acceptable. There are other considerations which are part of an evaluation of the lubricant effect of medicinals. Foremost among these is the ability of the lubricant to remain on the surface of the instrument to which it is applied, rather than to be removed mechanically or to be diluted by mucus.

The lack of correlation between viscosity and lubricant effect of the preparations tested seems to indicate that viscosity by itself is no criterion of the effectiveness of a lubricant. It is conceivable, however, that for each particular lubricant a viscosity range may exist within which the lubricant effect is at its maximum.

TABLE II.—FRICTION VALUES OF TRAGACANTH JELLY, 1.5%, 57,000 c. p. s.[a]

Sheet	Series I, Gm.	Series II, Gm.	Series III, Gm.
A	181	170	177
B	170	176	176
C	173	182	177
D	169	166	170
E	170	176	172
F	176	172	179
Average	173	174	175

[a] Brookfield viscometer, model LVF, spindle No. 4, 6 r. p. m. at 25°.

SUMMARY

1. An instrument for the determination of the lubricating property of medicinals has been described.

2. Experimental values for the lubricant effect of a few representative medicinal products have been presented.

3. The limitations as well as the significance of the instrument have been discussed.

REFERENCES

(1) Fuller, D. D., "Theory and Practice of Lubrication for Engineers," John Wiley & Sons, Inc., New York, 1956, pp. 346, 363.

(2) Burwell, J. T., and Strang, C. D., *J. Appl. Phys.*, **20**, 79(1949).

(3) Davies, C. B., *Ann. N. Y. Acad. Sci.*, **53**, 921(1951).

Viscosity Changes of Sodium Alginate Solutions after Freezing and Thawing*

By T. W. SCHWARZ and GERHARD LEVY

Pharmaceutical products containing sodium alginate are sometimes subjected to temperatures below freezing during storage or shipping. Freezing of sodium alginate solutions results in a viscosity increase which may impair their usefulness. The effects of additives, as well as variations in the method of preparation, on the viscosity of sodium alginate solutions were examined.

SODIUM ALGINATE is the sodium salt of alginic acid, which is a linear polymer of anhydro-β-D-mannuronic acid of high molecular weight (1). This substance is used in pharmaceuticals, cosmetics, foods, and other products as a thickening, stabilizing, emulsifying, and suspending agent (2, 3).

Since products containing sodium alginate may be subjected to temperatures below freezing during storage or shipping, we deemed it important to establish any possible influence of freezing on the properties of such products. The thickening ability of sodium alginate being its most useful property, this paper concerns itself with viscosity changes subsequent to freezing.

EXPERIMENTAL

Solutions of a type of sodium alginate[1] were prepared by sprinkling the required amount of the powder on distilled water agitated by a Waring Blendor. Any other component of the solution was predissolved in the water. The solution was subjected to a definite rate of shear for ten minutes. The now homogeneous solution was placed in 50-ml. centrifuge tubes and any bubbles of occluded air were removed by centrifuging.

The temperature of the solutions was adjusted to 25° ± 0.5° in a water bath. After twenty-four hours the viscosity was determined, using a Brookfield Synchro-Lectric Viscometer, model LVF. A No. 4 spindle was used for viscosities below 100,000 c. p. s., and a No. 5 spindle was used for viscosities above 100,000 c. p. s. The measurements were carried out at 6 r. p. m. after ten minutes of spindle rotation. The samples were then placed in a freezer at −16° ± 1° for twenty-four hours. After removal from the freezer, the samples were transferred to a water bath at 25° where they remained for twenty-four hours before their viscosity was again determined.

RESULTS AND DISCUSSION

Table I shows the viscosity changes of the various samples as a result of freezing and thawing. According to these data it may be stated that: (a) Sodium alginate solutions that have undergone freezing and thawing show a considerable viscosity increase. (b) The lower the initial concentration, and thus the viscosity of the solution, the greater is the relative viscosity increase. (c) The inclusion of glycerin or propylene glycol in the solution results in a markedly higher initial viscosity compared to solutions of equal concentration of sodium alginate in water alone. (d) The presence of propylene glycol or glycerin in sodium alginate solutions causes a smaller or no viscosity increase after freezing and thawing. The higher the concentration of these agents, the smaller is the relative viscosity increase after freezing. (e) Sodium alginate solutions containing calcium lactate in the concentrations tested

TABLE I.—VISCOSITY CHANGE OF SODIUM ALGINATE SOLUTIONS AFTER FREEZING AND THAWING

Composition of Sample, %	Av. Viscosity Before Freezing[a] (c. p. s.)	Av. Viscosity 24 Hr. After Freezing[a] (c. p. s.)	Ratio (Viscosity After / Viscosity Before)	No. of Samples
1.5 Sodium alginate	2,500	33,200	13.3	4
1.75 Sodium alginate	7,000	48,500	6.9	4
2.0 Sodium alginate	17,600	59,000	3.4	4
1.75 Sodium alginate with 10 glycerin	11,600	22,400	1.9	3
1.75 Sodium alginate with 20 glycerin	23,300	27,800	1.2	3
1.75 Sodium alginate with 10 propylene glycol	29,000	53,300	1.8	2
1.75 Sodium alginate with 20 propylene glycol	111,200	116,500	1.0	3
1.0 Sodium alginate with 0.1 calcium lactate	39,150	40,100	1.0	2

[a] Readings taken at 25°.

* Received March 6, 1957, from the University of California School of Pharmacy, San Francisco.
This study was conducted in part under a contract with the Armed Services Medical Procurement Agency.
[1] Kelcosol, Kelco Company.

show a negligible viscosity change after freezing and thawing.

The viscosity of sodium alginate solutions was determined over a period of fourteen days, while the solutions were kept at 25°. The results for samples which had been frozen and for those which had not been frozen are shown in Table II. The data indicate that the samples with a history of freezing retain a considerably higher viscosity than the nonfrozen controls even after fourteen days at room temperature. However, there is a gradual viscosity decrease of the frozen samples with time. To eliminate the effects of possible spoilage on the viscosity, the solutions were preserved with methylparaben (0.1%) and propylparaben (0.025%). The same experiment was carried out without preservatives and gave very similar results.

We have checked the effect of freezing on a proprietary lubricant (K-Y Jelly, Johnson and Johnson) which contains sodium alginate (4). This preparation showed an almost twofold viscosity increase after freezing.

TABLE II.—VISCOSITY CHANGES OF SODIUM ALGINATE SOLUTIONS STORED AT 25°. INITIAL VISCOSITY 7,100 C. P. S.[a]

	1 Day	7 Days	14 Days
Solutions after freezing and thawing	37,000	29,500	29,000
Nonfrozen solutions	7,000	6,500	6,100

[a] The figures represent the average of three solutions of 1.4%. A different batch of sodium alginate with a slightly higher thickening capacity was used in this experiment. Viscosity measurements at 25°.

The viscosity increase was not influenced by the length of time during which the samples stayed frozen. Solutions of equal viscosity, exposed to freezing from one to six days, showed the same increase of viscosity. Alginate solutions which did not freeze at temperatures slightly below zero, due to the inclusion of glycerin or propylene glycol, did not show the characteristic viscosity increase of frozen solutions. The same solutions, subjected to temperatures low enough to cause freezing, however, exhibited the marked viscosity increase reported here.

TABLE III.—THE INFLUENCE OF THERMAL AND MECHANICAL TREATMENT ON THE VISCOSITY OF 2% SODIUM ALGINATE SOLUTIONS BEFORE AND AFTER FREEZING AND THAWING

	Viscosity Before Freezing[a]	Viscosity After Freezing[a]
10-min. shear	17,600	59,000
60-min. shear	9,000	59,300
10-min. shear followed by 60-min. heating at 100°	3,200	30,300

[a] Average of 3 samples; readings taken at 25°.

The viscosity of sodium alginate solutions depends on several factors, among which are concentration and length of shear used in the course of their preparation. Solutions which were subjected to long *periods* of shear became considerably less viscous (Table III). Regardless of the difference in initial viscosity which resulted from differences in length of shear, all solutions of equal concentrations reached the same viscosity after freezing and thawing. The decrease of viscosity due to length of shear should be differentiated from the viscosity decrease due to increased *rate* of shear, a characteristic of pseudoplastic materials. It should also be differentiated from thixotropy, which, by definition, is an isothermal, reversible viscosity change (5). The high viscosity that resulted from freezing and thawing of the sodium alginate solutions could be reversed to the initial low viscosity by strong shearing. The solutions thus obtained showed no viscosity increase on standing, whereas thixotropic solutions do (Table IV).

TABLE IV.—EFFECT OF SHEAR ON THE VISCOSITY[a] OF FROZEN AND THAWED SODIUM ALGINATE SOLUTIONS (1.4%)

Before Freezing	1 Day After Freezing	After Shearing of the Frozen and Thawed Solution		
		1 Day	5 Days	10 Days
7,000	36,000	5,000	5,000	5,000

[a] Viscosity in c. p. s. All measurements at 25°.

The effect of heat on the viscosity was also investigated. The samples were heated for one hour in a water bath at 100°. The results are shown in Table III. Unlike the alginate solutions which were subjected to prolonged shearing before being frozen, the viscosity of the heat-treated solutions was considerably less after freezing than that of the unheated solutions. Heating causes degradation of the polymer chains (6) which results in lower viscosities both before and after freezing. One may assume, therefore, that the reversible viscosity decrease after prolonged shearing is due to a different effect on the polymer than the irreversible viscosity decrease after heating. These observations, as well as the results of additional work in progress, may help in gaining a better understanding of the nature of the internal structure of sodium alginate solutions.

SUMMARY

1. The viscosity of solutions of sodium alginate used in this investigation is increased after freezing and thawing.

2. The relative increase of viscosity depends upon the concentration of sodium alginate and the method used for the preparation of the solution.

3. The effects of glycerin, propylene glycol, and calcium lactate on the viscosity of solutions of sodium alginate before and after freezing are shown.

REFERENCES

(1) Steiner, A. B., and McNeely, W. H., in "Natural Plant Hydrocolloids," American Chemical Society, Washington, D. C., 1954, p. 68.
(2) Anon., "Algin at Work," Kelco Company, 1952.
(3) Lesser, M. A., *Drug & Cosmetic Ind.*, **61**, 761–762, 842(1947).
(4) "Remington's Practice of Pharmacy," 11th Ed., The Mack Publishing Company, 1956, p. 1031.
(5) Fischer, E. K., "Colloidal Dispersions," John Wiley & Sons, Inc., 1950, p. 182.
(6) Perlin, A. S., *Can. J. Chem.*, **30**, 278(1952).

Studies with a Uterine Antispasmodic, Ambucetamide*

By J. B. HOEKSTRA, DOROTHEA S. FISHER, K. M. CULL, D. E. TISCH, and H. L. DICKISON

Ambucetamide, 2-(di-*n*-butylamino)-2-(4-methoxyphenyl)-acetamide, has been found to have antispasmodic action on the uterus. Direct comparison of ambucetamide and papaverine on the same isolated, rat-uterine preparation indicated that they possessed activity of the same magnitude. On intact rat-uterine preparations, ambucetamide caused a decrease in the amplitude and frequency of contractions with an elevation in tone in about half the animals while the other half showed no effect. In the majority of the dogs studied, ambucetamide reduced the tone of the uterus and increased the amplitude of contractions. Uterine tone was also decreased following oral administration. The tone of the dog ileum was decreased following an intravenous dose of 5.0 mg./Kg. and type III contractions frequently appeared. A moderate decrease in blood pressure persisting for about one-half hour was also observed at this dose. Chronic toxicity studies were carried out in both rats and dogs. None of these animals developed any functional abnormalities nor did they show any gross or microscopic pathology at autopsy.

DEJONGH AND CO-WORKERS (1) have reported briefly on some pharmacological properties of 2-dialkylamino-2-phenyl-acetamides. They indicated that one of these compounds, 2-(di-*n*-butylamino)-2-(4-methoxyphenyl)-acetamide, which they designated A-16, had activity equal to that of papaverine on isolated intestinal strips, but was considerably less toxic. Later Carpentier and Janssen (2) investigated the effectiveness of this compound in dysmenorrhea and found a statistically significant favorable effect of A-16 when compared with placebo treatment.

This investigation was undertaken in order to obtain a better understanding of the pharmacological actions of this compound and to gain some knowledge as to its limitations so far as toxicity is concerned. A preliminary report on these studies has been presented by Hoekstra, *et al.* (3). The structure of this compound is shown in Fig. 1. Its chemistry has been described by Janssen (4). It has been given the generic name of ambucetamide.

EXPERIMENTAL

Results in vitro.—The effect of ambucetamide on the isolated rat uterus was studied by a modification of the method of Magnus (5). The isolated uterus was suspended in a Ringer Tyrode solution modified according to Smith (6) and maintained at 37.5°. In each case ambucetamide was left in the chamber for ten minutes before washing. In addition, the minimum concentration of papaverine which was required to relax the rat uterus was also determined on each preparation. Expressing this concentration of papaverine as unity, it was possible to determine a papaverine index for ambucetamide. Six to eight determinations of the ambucetamide effect were carried out on each strip. Two determinations of the papaverine effect were carried out on each strip. A total of eight different rat-uterine strips were employed.

Ambucetamide produced a decrease in the amplitude of contraction starting in most instances at a bath concentration of 0.025 mg./cc. This effect was quite marked at a concentration of 0.05 mg./cc. It produced a marked decrease in the frequency of contractions starting with a bath concentration of 0.125 mg./cc. No decrease in tone was noted at concentrations less than 0.025 mg./cc. However, starting at a concentration of 0.125 mg./cc. there was a definite drop in tone, as seen in Fig. 2, with complete inhibition of activity occurring at a concentration of 0.5 mg./cc. Occasionally at concentrations less than 0.025 mg./cc., an increase in tone and frequency and a decrease in the amplitude of contractions was seen. Ambucetamide was found to have a papaverine index of 1.0.

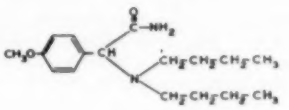


Figure 1.

Action on Uterus in situ.—These studies were carried out in both rats and dogs anesthetized with sodium pentobarbital. In the rat the uterus was carefully separated from the ovarian ligament and attached by means of a thread to a recording lever as described by Barbour (7). An internal organ recording device as described by Jackson (8) was used for the dog uterine studies. Thirty-nine rats and eighteen dogs were used in this study. The drug was administered to the rats by way of the jugular vein and to the dogs by way of the femoral

* Received March 28, 1957, from the Research Division, Bristol Laboratories Inc., Syracuse, N. Y.

The authors wish to thank Dr. W. Walter Street, State University of New York, College of Medicine, Syracuse, and Dr. Howard Albright, Bristol Laboratories, Inc., for their aid in interpreting the electrocardiographs; and Dr. Martin F. Hilfinger, Jr., and Dr. Carl I. Austin, Department of Pathology, State University of New York, College of Medicine, Syracuse, for their examination of the sections of the animal tissues removed at autopsy.

TABLE I.—EFFECT OF AMBUCETAMIDE ON RAT UTERUS IN SITU

Dose, mg./Kg.	No. of Rats	Tone	Frequency of Contractions	Amplitude of Contractions
0.1	2	1 rat—delayed drop 1 rat—slight rise	1 rat—decrease 1 rat—no change	1 rat—initial increase with a delayed decrease. 1 rat—slight decrease
0.5	3	1 rat—no change 1 rat—delayed drop 1 rat—slight rise	2 rats—slight decrease 1 rat—delayed decrease	3 rats—no change
1.0	6	4 rats—no change 1 rat—slight rise 1 rat—persistent rise	3 rats—no change 3 rats—slight decrease	2 rats—no change 3 rats—decrease 1 rat—slight decrease followed by complete inhibition
5.0	7	4 rats—no change 3 rats—initial rise	4 rats—no change 3 rats—slight decrease	6 rats—decrease (delayed) 1 rat—no change
10.0	8	4 rats—no change 1 rat—drop 3 rats—initial rise with two showing a secondary delayed drop	5 rats—no change 1 rat—decrease followed by inhibition. 2 rats—decrease	5 rats—no change 3 rats—decrease
20.0	12	5 rats—initial drop with two showing a persistent drop 4 rats—rise with one delayed 2 rats—persistent rise 1 rat—initial rise	8 rats—no change 4 rats—decrease with one showing complete intermittent inhibition	6 rats—no change 1 rat—fewer large contractions 3 rats—decrease 2 rats—increase
30.0	1	Persistent rise	Decrease	No change

vein. Solutions for testing were obtained by adding a known weight of base to water and adding concentrated hydrochloric acid dropwise until solution occurred. In addition to the above dogs, two others received ambucetamide orally.

Rats.—The results of these studies in rats are shown in Table I. There was no effect on the uterus in slightly less than half the animals at doses of 0.1 through 10.0 mg./Kg. At a dose of 20.0 mg./Kg. about one-half of the animals showed an increase and the other half showed a decrease in tone.

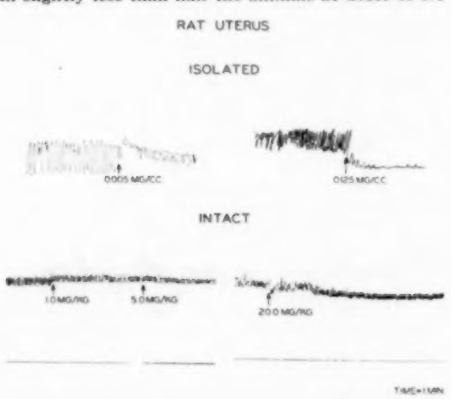


Fig. 2—Effect of ambucetamide on the rat uterus. Upper left—increase in tone and frequency and decrease in amplitude of contractions of isolated rat uterine preparation following a dose of 0.005 mg./cc. of ambucetamide. Upper right—decrease in tone and inhibition of activity of isolated rat uterus after a dose of 0.125 mg./cc. of ambucetamide. Lower left—slight increase in tone of intact rat uterus, following a dose of 1.0 mg./Kg. of ambucetamide; and a slight decrease in the amplitude of contractions, following a dose of 5.0 mg./Kg. Lower right—decrease in tone and slight decrease in amplitude of contractions of the intact rat uterus following a dose of 20.0 mg./Kg. of ambucetamide.

There was also noted a decrease in the amplitude and frequency of uterine contractions in about one-half of the rats studied while the remainder showed no change. Examples of these effects are shown in Fig. 2.

Dogs.—In general, doses of 1.0 to 10.0 mg./Kg. of ambucetamide produced a drop in tone in the majority of the animals and had no effect upon the frequency of contractions. In about 50% of the dogs there was an increase in the amplitude of contractions at doses of 1.0 and 5.0 mg./Kg., but at a dose of 10.0 mg./Kg. the majority showed a decrease in the amplitude of contractions. Ambucetamide did not exhibit a significant effect on the uterus of every dog, which parallels the findings in the rat studies. Figure 3 shows the types of responses obtained.

Following oral administration of a dose of 50 mg./Kg. of ambucetamide to one dog there was a gradual, moderate decrease in tone with a very slight decrease in the amplitude of contractions. After a dose of 100 mg./Kg. in another dog there was a marked drop in tone within one-half hour. These effects persisted for at least five hours (Fig. 3).

The effect of ambucetamide on the gravid uterus, as noted in several dogs, is of special interest. In these animals the compound caused a decrease in the tone of the uterus and a decrease in the duration of the contractions (Fig. 4).

One dog which had been placed under pentobarbital anesthesia began to deliver her pups spontaneously. After one pup had been delivered, the dog's abdomen was quickly opened and an internal organ recorder was attached to the body of the uterus. The uterus at this time was in a state of massive contraction. A dose of 10.0 mg./Kg. of

ambucetamide was then given intravenously. The uterus immediately relaxed and no further evidence of return to the state of massive contraction was noted during the rest of the day.

The effect of ambucetamide on Pitocin-induced uterine spasms was studied in two dogs anesthetized with sodium pentobarbital. In these dogs intravenous doses of 10.0 mg./Kg. did not prevent the spasms induced by doses of 0.5, 1.0, and 5.0 units/Kg. of Pitocin given intravenously.

Action on Intestinal Tract.—Dogs were placed under pentobarbital anesthesia and the terminal ileum was attached to an internal organ recorder (8). Following this, intravenous ambucetamide was given to these animals by way of the femoral vein. Doses ranging from 0.1 to 20.0 mg./Kg. were given stepwise to each animal with a minimum time interval of one-half hour between each dose. Seven dogs were used in this study.

Little, if any, effect on the ileum was noted in these dogs at doses less than 1.0 mg./Kg. Beginning with a dose of 5.0 mg./Kg., most of the animals showed a decrease in tone of the ileum. Large tone waves, type III contractions, were seen frequently following doses of 1.0 to 10.0 mg./Kg. of ambucetamide (Fig. 4).

Blood Pressure Studies.—These studies were carried out on dogs anesthetized with sodium pentobarbital. Blood pressure was recorded from the carotid artery by means of a mercury manometer and injections were made into the femoral vein. After determining the dog's normal responses to histamine diphosphate (0.003 mg./Kg.), acetylcholine chloride (0.003 mg./Kg.), and epinephrine hydrochloride (0.001 mg./Kg.); a dose of 0.1 mg./Kg. of ambucetamide was given. The responses to the above test drugs were again determined. This procedure was repeated after each stepwise increase in the dose of ambucetamide. A suitable interval of time was allowed between administrations of all drugs to allow the blood pressure to assume a steady state. Nine dogs were used in this study.

All of the effects on blood pressure, elicited either by ambucetamide or the test drugs, were measured in millimeters of mercury pressure and expressed as a per cent of the existing blood pressure, prior to injection. The average response to the test drugs at the beginning of the experiment served as a standard of comparison for the responses obtained after the administration of ambucetamide. These responses were expressed as a per cent of the original average response in order to indicate the changes which might be attributed to ambucetamide.

The mean effects of different doses of ambucetamide on blood pressure and on the vascular responses to the test drugs are shown in Table II.

TABLE II.—EFFECT OF INTRAVENOUS AMBUCETAMIDE ON BLOOD PRESSURE AND ON ALTERATIONS IN BLOOD PRESSURE FOLLOWING INJECTION OF HISTAMINE, ACETYLCHOLINE, AND EPINEPHRINE

Dose, mg./Kg.	Fall in Blood Pressure, %	Per cent of Normal Response		
		Histamine, 0.003 mg./Kg.	Acetylcholine, 0.003 mg./Kg.	Epinephrine, 0.001 mg./Kg.
0.1	0[a]	109.0[b]	84.3[a]	102.1[b]
0.5	3.3[a]	108.6[b]	77.8[a]	127.1[b]
1.0	11.7[a]	106.9[b]	89.1[a]	116.0[c]
5.0	26.6[a]	117.5[a]	68.8[a]	120.9[a]
10.0	44.6[a]	100.5[a]	80.7[b]	135.2[c]
20.0	66.7[c]	94.8[d]	95.8[d]	138.0[d]

[a] Average of results from eight animals.
[b] Average of results from seven animals.
[c] Average of results from six animals.
[d] Average of results from four animals.

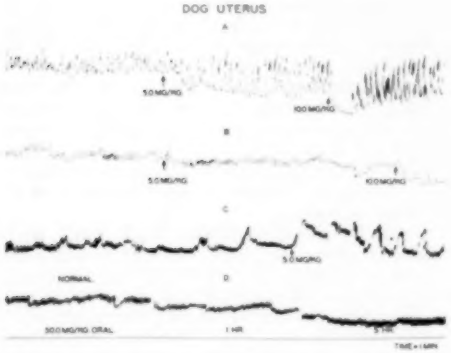


Fig. 3—Effect of ambucetamide on the dog uterus. A.—Decrease in tone and increase in amplitude of contractions, following doses of 5.0 and 10.0 mg./Kg. of ambucetamide. B.—Decrease in tone and slight decrease in amplitude of contractions at a dose of 5.0 mg./Kg.; and decrease in tone, and almost complete inhibition of activity, following a dose of 10.0 mg./Kg. C—Rise in tone following a dose of 5.0 mg./Kg. D—Decrease in tone and slight decrease in amplitude of contractions following an oral dose of 50.0 mg./Kg. Tone is lower than normal after one hour and is much lower at five hours after administration of ambucetamide.

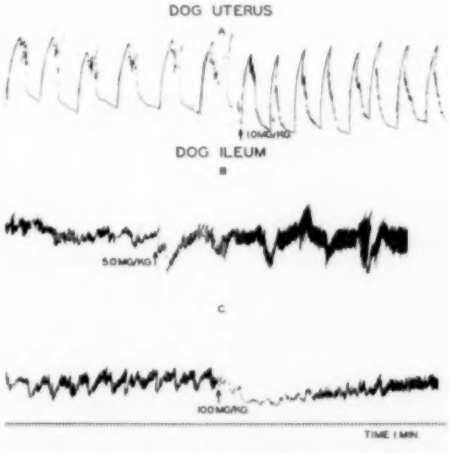


Fig. 4—Effect of ambucetamide on gravid uterus and ileum. A—Decrease in tone and duration of large spasm-like contractions of gravid uterus near term by 1.0 mg./Kg. of ambucetamide. B—Decrease in tone of ileum after a dose of 5.0 mg./Kg. of ambucetamide and initiation of Type III contractions. C—Decrease in tone and activity of ileum following a dose of 10.0 mg./Kg.

These results show that ambucetamide produced a depressor response on blood pressure beginning at a dose of approximately 1.0 mg./Kg. This response occurs immediately and then starts to return to normal quite rapidly after a dose of 1.0 mg./Kg., but requires an average of 33, 37, or 55 minutes after doses of 5.0, 10.0, and 20.0 mg./Kg., respectively.

Ambucetamide had no significant effect on the responses to histamine, but slightly reduced the responses to acetylcholine and slightly increased the responses to epinephrine.

Effect on the Heart (Electrocardiographic Studies).—Stepwise intravenous doses of 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, and 40.0 mg./Kg. of ambucetamide were given to five dogs anesthetized with sodium pentobarbital and electrocardiograms were recorded. A thirty-minute interval elapsed between each of the above doses. At doses of 0.1 through 10.0 mg./Kg. there were no significant changes in the electrocardiogram, with the exception of a slight slowing of the heart and minor T wave changes at doses of 5.0 and 10.0 mg./Kg. These changes were transient.

Ambucetamide produced a marked myocardial depression at a dose of 20.0 mg./Kg. During the ensuing thirty minutes after the injection, this depression which occurred immediately became less severe and returned slowly toward a normal pattern. Sufficient time for recovery was not allowed since a dose of 40.0 mg./Kg. was given at this time. This latter dose always produced a severe myocardial depression and death occurred in every instance.

Action on Respiration.—Respiration was recorded kymographically on dogs anesthetized with sodium pentobarbital by means of a tracheal cannula attached to a tambour. Most dogs showed an increase in rate and amplitude of respiration at doses of 5.0 through 20.0 mg./Kg.

Action on Salivary Glands.—The effect of ambucetamide on the salivary secretion induced by 0.1 mg./Kg. of pilocarpine hydrochloride was studied in two dogs anesthetized with sodium pentobarbital. No blocking of this secretion was noted in either dog at doses through 20.0 mg./Kg.

TOXICITY

Acute Toxicity in Mice.—These studies have been carried out in both mice and dogs. The ambucetamide was suspended in water and sufficient hydrochloric acid added to cause solution to occur. The results of these studies in mice are presented in Table III. A calculation of the LD_{50} was done by the deBeer graphic method (9).

The intravenous LD_{50} in Table III is in good agreement with the value of 63 ± 6 mg./Kg. reported by DeJongh, *et al.* (1).

Acute Toxicity in Dogs.—The intravenous toxicity of ambucetamide was studied in a total of nine unanesthetized dogs. Three dogs received rapid intravenous doses of 5.0 mg./Kg. and exhibited symptoms ranging from apprehension and extreme nervousness to a mild state of tetany. All of these effects were transient and the animals appeared normal again after a period of about five minutes. Two of these dogs received an additional dose of 5.0 mg./Kg. after a lapse of 10 to 15 minutes and exhibited a reaction either equal to or less severe than that occurring after the first dose. Daily administration of this dose for three days to the third dog resulted in a progressive decrease in the reaction exhibited by this animal. Six dogs received intravenous doses of 10.0 mg./Kg. of ambucetamide. All of these dogs were quite apprehensive immediately after the injection and three of them developed a mild state of tetany. All of these dogs had returned to an apparently normal state within fifteen minutes after receiving this dose of ambucetamide.

Six unanesthetized dogs received oral doses of 100.0 mg./Kg. of ambucetamide. This was administered by stomach tube in the form of a 1% solution. All of the dogs developed a mild state of tetany from which they recovered uneventfully. These same dogs tolerated a dose of 50.0 mg./Kg. without any untoward effects.

In anesthetized dogs successive intravenous doses of 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, and 40.0 mg./Kg. of ambucetamide were given with an approximate time interval of one-half hour between doses. Thirteen dogs received the successive doses up through 20.0 mg./Kg. Only two deaths occurred after the 20.0 mg./Kg. dose (cumulative 36.6 mg./Kg.). Six dogs received the 40 mg./Kg. dose and all died after this dose (cumulative 76.6 mg./Kg.). Oral doses of 50.0 and 100.0 mg./Kg., respectively, to two anesthetized dogs were not lethal.

Chronic Toxicity in Rats.—Thirty weanling female rats were given an oral dose of 30 mg./Kg. in the form of a 1% solution of ambucetamide. A similar group of thirty female rats received a dose of 60.0 mg./Kg. A third group of twenty female weanlings served as controls and received a dose of water containing dilute hydrochloric acid in a volume equal to that necessary to contain the 60.0 mg./Kg. dose. The rats were weighed and dosed daily, except on Saturday and Sunday. The solutions were administered by means of an oral feeding needle attached to a hypodermic syringe. The dose was adjusted daily according to the weight of the animal. The duration of this experiment was thirteen weeks.

Two rats in the 60.0 mg./Kg./day group began to show traces of blood in the urine after three weeks; therefore the dose was decreased to 50 mg./Kg./day. One rat from each of the two different dose schedules of the drug was sacrificed weekly, starting with the fourth week, and a post-mortem examination was performed. At the end of thirteen weeks all the remaining rats were sacrificed by decapitation and post-mortem examinations were carried out. The gross findings at autopsy in all cases were negative and resembled those found in the controls. Sections of the thymus, lung, heart, liver, spleen, stomach, adrenal, kidney, ovary, and uterus were removed and prepared for microscopic examination. No microscopic abnormalities were reported.

There were no deaths during the course of the ex-

TABLE III.—ACUTE TOXICITY—MICE

Method of Administration	Solution	LD_{50} mg./Kg.	Slope	No. Mice
Intraperitoneal	2% aqueous	91.85 ± 4.1	33	55
Intravenous	0.8% aqueous	62.2 ± 2.1	40	15
Oral	2% aqueous	813 ± 45.5	17	97

periment and the animals appeared healthy at all times. Weight gains in all three groups of rats were comparable throughout the experiment. The gain in weight expressed as a per cent for the three groups finishing the experiment was: Controls (20 females) 352.8%; 30 mg./Kg. group (20 females), 357.7%; and the 50 mg./Kg. group (20 females), 340.0%.

DeJongh, *et al.* (1), reported that weanling rats tolerated 100 mg./Kg. of this material by stomach tube for a period of two months.

Chronic Toxicity in Dogs.—A total of nine female dogs received various daily doses of ambucetamide for varying periods of time. All dogs were dosed orally. The crystalline material was placed in gelatin capsules for this purpose. The doses were adjusted according to the animals' weights at weekly intervals.

Eight dogs were given doses of 100.0 mg./Kg. for periods ranging from one to three weeks. One of these dogs had the dose raised to 200 mg./Kg. for one week, followed by a dose of 150 mg./Kg. for the next five weeks, and during the next forty-five weeks was given 50.0 mg./Kg. A second dog had the dose dropped to 75.0 mg./Kg. for five weeks, and after that was dosed at 50.0 mg./Kg. for forty-five weeks. After the initial period of dosing with 100.0 mg./Kg., the other six dogs were maintained on a dose of 50.0 mg./Kg. during the remainder of the one-year study. At doses in excess of 50.0 mg./Kg. all the dogs experienced difficulty in maintaining their weight. At these doses the animals tended to become lethargic and all suffered some loss of appetite. At doses of 50.0 mg./Kg. all animals ate well and appeared normal. Those animals which had lost weight on the higher doses gained back their weight. Complete studies of blood chemistry, urinalysis, and blood counts were performed on four of these dogs before starting the experiment and throughout the year. No significant deviations from normal were seen in any of the dogs. Kidney function as determined by the phenolsulfonphthalein test was normal throughout in these same four dogs. Liver function as determined by the bromsulfalein test was also normal in all of the eight dogs.

One of the dogs became pregnant during the course of this study and delivered a litter of six healthy pups. Another experiment was carried out in order to determine whether ambucetamide had any adverse effect on pregnancy and parturition. A dog was bred and after it was certain that the animal was pregnant she was dosed with 50 mg./Kg./day of ambucetamide. At term she delivered seven healthy pups over a twenty-four hour period. Bromsulfalein tests on this dog were likewise normal throughout the experiment.

All dogs, with the exception of the last one described, were sacrificed at the end of the experiment and post-mortem examinations were performed. No gross or microscopic pathologic changes which could be attributed to a toxic action of ambucetamide were found.

SUMMARY

1. Ambucetamide, 2 - (di - *n* - butylamino) - 2-(4-methoxyphenyl)-acetamide, has been found to have a relaxant effect on the uterus of both rats and dogs equal to that of papaverine.

2. Ambucetamide does not prevent conception or seriously interfere with the progress of gestation and parturition.

3. Ambucetamide has a relaxant effect on intestinal smooth muscle.

4. Ambucetamide has a slight depressor effect on blood pressure at high doses and has little effect on the vascular responses of autonomic drugs.

5. Ambucetamide is relatively nontoxic and is well tolerated on chronic administration in both rats and dogs. Unanesthetized dogs tolerated intravenous doses of 10.0 mg./Kg. and anesthetized dogs tolerated 20.0 mg./Kg. Daily oral doses of 50.0 mg./Kg. have been well tolerated in dogs for a year and have produced no untoward effects. Rats have tolerated similar oral doses for three months with no adverse effect on their growth rate.

REFERENCES

(1) DeJongh, D. K., Janssen, P. A., and Van Proosdij-Hartzema, E. G., *Acta Physiol. et Pharmacol. Neerl.*, **3**, 331 (1954).
(2) Carpentier, P. J., and Janssen, P. A., *Lancet*, **1**, 122 (1955).
(3) Hoekstra, J. B., Fisher, Dorothea S., Tisch, D. E., and Dickison, H. L., *J. Pharmacol. Exptl. Therap.*, in press.
(4) Janssen, P. A., *J. Am. Chem. Soc.*, **76**, 6192(1954).
(5) Magnus, R., *Arch. ges. Physiol.*, **102**, 123(1904).
(6) Solomon, Torald H. and Hanzlik, Paul J., "Fundamentals of Experimental Pharmacology," 2nd ed., J. W. Stacey, Inc., San Francisco, Calif., 1939, p. 295.
(7) Barbour, H. G., *J. Pharmacol. Exptl. Therap.*, **7**, 547 (1915).
(8) Jackson, D., "Experimental Pharmacology and Materia Medica," 2nd ed., The C. V. Mosby Co., St. Louis, Mo., 1939, p. 89.
(9) deBeer, E. J., *J. Pharmacol. Exptl. Therap.*, **94**, 7 (1948).

Notes

A Note on the Effect of Boric Acid and Sodium Metaborate on the Oxygen Uptake of Brain Tissue*

By ALFRED S. C. LING, EDWARD B. TRUITT, JR., and JOHN C. KRANTZ, JR.

THE TOXIC EFFECTS of boric acid and other boron compounds have been described by various investigators [Pfeiffer, Hallman and Gersh (1), Pfeiffer and Jenny (2), and Lang (3)]. In borate poisoning the symptoms may include convulsive tremors, lethargy, and coma. Furthermore, Mcnally and Rust (4) and others observed that boric acid is concentrated in the central nervous system, especially the gray matter of the brain. The present work was undertaken to study the effect of boric acid and metaborate on the oxygen utilization of brain tissue from rats and guinea pigs *in vitro*.

METHODS AND MATERIALS

Male albino rats and guinea pigs were sacrificed by decapitation. The brains were promptly placed in cold Krebs-Ringer-phosphate medium. Cerebral-cortical slices, approximately 0.5 mm. thick, were used for study. For the homogenate studies 10% suspensions of various sections of the brain were prepared using a Potter-Elvehjem homogenizer. The oxygen uptake of the brain tissue was measured by the direct technic. The medium was Krebs-Ringer-phosphate, pH 7.4; the substrate, when present, was either 0.2% glucose (0.011 *M*) or 0.1% sodium pyruvate (0.01 *M*). The gas phase was oxygen for tissue slices and air for homogenates. After a ten-minute equilibration period readings were recorded every ten minutes for one to two hours. The $-Q_{O_2}$ values represent μl. O_2 consumed per mg. final, dry weight per hour. In general, the borates were added directly to the flask at the beginning of each experiment. Two or more control flasks were run concurrently with the borate-treated flasks.

TABLE I.—CONTROL $-Q_{O_2}$ VALUES OF RAT CEREBRAL CORTICAL SLICES

	Mean (Range)	No. of Determinations	S.D.[a]		P
$-Q_{O_2}$ based on $^1/_2$-hr. interval	12.82 (10.51–15.37)	10			
			0.85	0.611	<0.70
					>0.50
$-Q_{O_2}$ based on full-hr. interval	12.99 (11.05–14.13)	10			

[a] Standard deviation of paired differences.

TABLE II.—EFFECT OF BORIC ACID ON RESPIRATION OF RAT CEREBRAL CORTICAL SLICES

Concn.[a] of boric acid	Substrate	No. of Determinations	$-Q_{O_2}$, Mean (Range) Control	$-Q_{O_2}$, Mean (Range) Experimental	Average % Inhibition
7.14×10^{-2} *M*	0.011 *M* glucose	2	12.38 (11.50–13.26)	10.38 (10.38, 10.38)	16.16
3.57×10^{-2} *M*	0.011 *M* glucose	5	12.85 (11.74–13.84)	10.95 (10.14–12.20)	14.79
7.14×10^{-3} *M*	0.011 *M* glucose	9	11.55 (10.85–12.15)	10.99 (9.46–12.24)	4.85
7.14×10^{-3} *M*	0.01 *M* sodium pyruvate	8	16.78 (14.77–18.80)	13.53 (12.29–15.30)	19.37

[a] Concn. in flask after dilution with medium.

RESULTS AND DISCUSSION

Boric acid does not appreciably depress the oxygen consumption of brain tissue in the presence of glucose or pyruvate (Table II). In the absence of substrate, however, a marked depression by boric acid or sodium metaborate prevailed (Tables IV and V). The degree of inhibition evoked by boric acid and sodium metaborate appears to be a function of concentration within the levels employed provided the medium was substrate-free. The inhibition of oxygen consumption elicited by boric acid showed no specificity for any of the four areas of the brain studied (Table VI). Brain tissue stimulated by either the potassium ion or α-dinitrophenol responded to boric acid in a manner similar to the untreated tissue (Table III).

* Received November 23, 1956, from the Department of Pharmacology University of Maryland School of Medicine, Baltimore.

TABLE III.—EFFECT OF BORIC ACID ON RESPIRATION OF RAT CEREBRAL CORTICAL SLICES AFTER STIMULATION

Stimulant	No. of Determinations	Q_{O_2}, Mean (Range) Control	Q_{O_2}, Mean (Range) Experimental	Average % Increase	Concn.[a] of Boric Acid	Q_{O_2}, Mean (Range) Control	Q_{O_2}, Mean (Range) Experimental	Average % Inhibition
2.68×10^{-3} *M* dinitrophenol	2	11.15 (9.65–12.35)	18.14 (16.64, 19.64)	62.69	3.57×10^{-2} M	23.93 (22.72–25.35)	19.87 (18.55–21.19)	16.97
7.14×10^{-2} *M* KCl	2	13.63 (–)	15.54 (15.34, 15.73)	14.01	7.14×10^{-2} *M*	15.54 (15.34–15.73)	14.40 (14.47–14.33)	7.34

Substrate used: 0.2% glucose (0.011*M*)

[a] Concn. in flask after dilution with medium.

TABLE IV.—EFFECT OF BORIC ACID ON THE RESPIRATION OF RAT BRAIN CORTICAL SLICES IN SUBSTRATE-FREE MEDIUM

Concn.[a] of Boric Acid	No. of Determinations	Q_{O_2}, Mean (Range) Control	Q_{O_2}, Mean (Range) Experimental	Average % Inhibition
7.14×10^{-2} *M*	6	5.89 (4.51, 7.27)	3.09 (2.22–3.96)	47.54
3.57×10^{-2} *M*	8	5.76 (5.03–6.67)	4.58 (4.22–5.18)	20.49
7.14×10^{-2} *M*	6	5.67 (5.03–6.67)	5.71 (5.04–6.24)	0.71

[a] Concn. in flask after dilution with medium.

TABLE V.—THE EFFECT OF SODIUM METABORATE ON THE OXYGEN UPTAKE OF RAT CEREBRAL CORTICAL SLICES IN SUBSTRATE-FREE MEDIUM

Concn.[a] of Sodium Metaborate	No. of Determinations	Q_{O_2}, Mean (Range) Control	Q_{O_2}, Mean (Range) Experimental	Average % Inhibition
7.14×10^{-2} *M*	6	6.82 (6.82, 6.82)	4.29 (3.54–5.16)	37.10
3.57×10^{-2} *M*	6	6.34 (5.62, 7.06)	4.88 (4.54–5.33)	23.03
1.78×10^{-2} *M*	6	6.56 (6.04, 7.08)	6.34 (5.77–6.96)	3.35

[a] Concn. in flask after dilution with medium.

TABLE VI.—THE RELATIONSHIP OF THE RESPIRATORY ACTIVITIES OF SEVERAL GUINEA PIG BRAIN FRACTIONS AND BORIC ACID EFFECT

Brain Homogenate Fraction	No. of Determinations	Q_{O_2}, Mean (Range) Control	Q_{O_2}, Mean (Range) 7.14×10^{-2} *M* Boric Acid	Q_{O_2}, Mean (Range) 3.57×10^{-2} *M* Boric Acid	Average % inhibition 7.14×10^{-2} *M* Boric Acid	Average % inhibition 3.57×10^{-2} *M* Boric Acid
Cerebrum	6	2.94 (2.79–3.09)	2.42 (2.34–2.50)	2.67 (2.56–2.87)	17.69	9.18
Cerebellum	6	1.89 (1.36–2.42)	1.50 (1.00–1.87)	1.65 (1.06–2.11)	20.62	12.70
Medulla oblongata	6	1.17 (1.02–1.35)	0.94 (0.89–1.04)	1.01 (0.93–1.07)	19.66	13.68
Thalamus-hypothalamus	6	2.08 (1.84–2.32)	1.80 (1.68–1.89)	1.90 (1.84–2.06)	13.46	8.65

Medium contained no substrate

According to Trautner and Messer (5), the inhibition of brain oxygen uptake by borate may possibly be due to a complex formation between the drug and the hexose available to the cell, thus leading to glucose exhaustion of the cell. However, these investigators assert that this phenomenon, apparently, is not one simply of borate detoxification through complex formation; for mannitol, which forms complexes with borate, does not inhibit the respiratory depression caused by borate. Furthermore, Pfeiffer, Hallman and Gersh (1) have used a 5% glucose solution without success as an antidote to boric acid poisoning. Other polyhydroxyl compounds found to be ineffective were sorbitol and mannitol. In more recent work, Messer and Trautner (6) suggest that the toxic effect of borate may be related to its inhibition of ammonia formation and glutamine synthesis in brain tissue.

SUMMARY

1. Boric acid, at the concentrations studied, exerts only a negligible depression of oxygen uptake by rat cerebral tissue when glucose or pyruvate substrate is present in the medium. In the absence of such substrate, boric acid and sodium metaborate markedly inhibited oxygen utilization.

2. Boric acid does not appear to act preferentially on the four areas of the rat brain studied, namely, the cerebrum, cerebellum, medulla oblongata, and hypothalamus-thalamus.

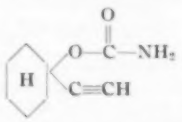
REFERENCES

(1) Pfeiffer, C. C., Hallman, L. F., and Gersh, I., *J. Am. Med. Assoc.*, **128**, 266(1945).
(2) Pfeiffer, C. C., and Jenny, E. H., *Bull. Nat. Formulary Comm.*, **18**, 57(1950).
(3) Lang, K., *Deut. Drogisten-Z.*, **5**, 565(1950).
(4) McNally, W. D., and Rust, C. A., *J. Am. Med. Assoc.*, **90**, 382(1928).
(5) Trautner, E. M., and Messer, M., *Australian J. Biol. Sci.*, **6**, 501(1953).
(6) Messer, M., and Trautner, E. M., *Australian J. Exptl. Biol. Med. Sci.*, **33**, 199(1955).

A Note on Crystallographic and X-Ray Diffraction Data for Ethinamate*

By ANN VAN CAMP† and R. R. PFEIFFER

ETHINAMATE, ethinylcyclohexylcarbamate, has the following structural formula:



It was first described as a hypnotic agent by Langecker, Schumann, and Junkmann (1) and studied clinically and pharmacologically by a number of other investigators (2–6). Data permitting the optical and x-ray crystallographic identification of the compound are presented herewith:

TABLE I.—UNIT CELL DATA FOR ETHINAMATE

Crystal system:	Orthorhombic
Unit cell dimensions:	a_0 = 15.41 Å.
	b_0 = 24.59 Å.
	c_0 = 9.81 Å.
Axial ratio:	0.6267:1:0.3990
Density:	1.187 Gm./cc. (flotation)
	1.197 Gm./cc. (x-ray)
Formula weights ($C_9H_{13}NO_2$) per cell:	16
Space group:	P_{bca}; D_{2h}^{15}

TABLE II.—OPTICAL CRYSTALLOGRAPHIC DATA FOR ETHINAMATE

Refractive indexes:	(5893 Å., 25°) α = 1.530, β = 1.536, γ = 1.546
Optic axial angle:	$2V$ = (+) 76°
Optic axial plane =	001
Acute bisectrix:	$\gamma = c$

EXPERIMENTAL

A sample of commercial "Valmid" (Ethinamate, Lilly) was recrystallized from ethanol-water to obtain crystals convenient for crystallographic study. The observed melting point was 98–99.5°.

The unit-cell data were obtained from Weissenberg photographs, which showed no systematic absences in *HKL*, but showed *OKL* present only when $K = 2n$, *HOL* present only when $L = 2n$, and *HKO* present only when $H = 2n$, thus establishing the space group as P_{bca}.

Powder diffraction data were obtained using V-filtered chromium radiation (λ = 2.2896 Å.) and a camera of 114.6 mm. diameter. The relative intensities were determined by visual comparison with standardized film exposures.

TABLE III.—X-RAY POWDER DIFFRACTION DATA FOR ETHINAMATE

d	I/I_1	hkl	d (calcd.)
12.4	0.13	020	12.3
7.90	0.27	111	7.84
6.53	0.21	220	6.53
5.62	0.05	230	5.61
5.44	0.13	221	5.44
5.23	0.67	041	5.21
4.90	1.00	231, 002	4.87, 4.90
4.61	0.13	112	4.59
4.38	0.08	122	4.37
4.27	0.05	321	4.27
4.17	0.01	250	4.16
4.07	0.27	132	4.06
3.96	0.02	222	3.92
3.83	0.10	410, 042, 251	3.81, 3.83, 3.82
3.72	0.05	420, 232	3.68, 3.69
3.67	0.02	341	3.66
3.54	0.02	411, 302	3.55, 3.55
3.48	0.05	421	3.44
3.40	0.08	322	3.41
3.34	0.08	351	3.34
3.26	0.02	440	3.26
3.19	0.02	270	3.20
3.10	0.05	441, 123	3.10, 3.10
3.03	0.13	412	3.01
2.91	0.01	261, 451	2.91, 2.90
2.85	0.05	280	2.86
2.72	0.05	442	2.72
2.58	0.05	253, 600	2.57, 2.57
2.55	0.02	381	2.55
2.51	0.05	620	2.51
2.44	0.05	462	2.44
2.37	0.01	.....	.....
2.28	0.01	.....	.....
2.17	0.02	.....	.....
2.11	0.01	.....	.....
2.070	0.02	.....	.....
2.033	0.01	.....	.....
1.994	0.01	.....	.....
1.968	0.01	.....	.....
1.939	0.01	.....	.....

REFERENCES

(1) Langecker, H., Schumann, H. J., and Junkmann, K., *Arch. Exptl. Pathol. Pharmakol.*, **219**, 130(1953).
(2) Tewes, H., *Ärztl. Praxis*, **6**, 1(1954).
(3) Schulz, K. H., and Schirren, C., *Deut. med. Wochschr.*, **79**, 1186(1954).
(4) Franke, G., *Med. Klin. (Munich)*, **49**, 891(1954).
(5) Foltz, E. L., Dracos, F., and Gruber, C. M., Jr., *Am. J. Med. Sci.*, **230**, 528(1955).
(6) Swanson, E. E., Anderson, R. C., and Gibson, W. R. THIS JOURNAL, **45**, 40(1956).

* Received December 14, 1956, from Lilly Research Laboratories, Indianapolis 6, Ind.

† The authors are indebted to Dr. H. A. Rose of these laboratories for the data in Table III.

A Note on Mathematics of Oral Sustained Release Products*

By EINO NELSON

DURING THE LAST FIVE OR SIX YEARS there have been a number of products marketed by pharmaceutical manufacturers as sustained release preparations and it is of interest to examine the mathematical factors involved in their design. This note briefly considers these factors and presents a simple scheme for estimating amounts of drug needed to meet requirements for sustained release.

THEORY

When drugs are administered orally, there are several processes determining persistence time, maximum level, and time to reach maximum concentration in the fluids of distribution. The rates of all processes are concentration dependent and may be described by the following scheme:

$$A \rightarrow B \rightarrow C \rightarrow D$$

Here A represents drug in solid form at the absorption sites; B, drug in solution at the absorption sites; C, drug in the fluids of distribution; and D, degraded or eliminated drug.

The rate of the first step, solid to solution, determines the time of onset of action and maximum concentration reached in the fluids of distribution, since the rate of all processes following are concentration dependent. Similarly, the rate of absorption affects the rate of processes following it, although in some cases it may be rapid enough to make the solid-to-solution step rate determining. For example, it has been calculated that this would be true in the case of aspirin being absorbed by free diffusion (1). The rate of the elimination process is first order in the majority of the cases studied. The mathematics of the elimination process has recently been reviewed (2).

In the following it will be assumed that the rate-determining step after the solid drug is at absorption sites in the solution rate in the fluids present at the sites, i.e., the rate of step B to C is much faster than A to B. Calculations made on this basis should not be greatly affected even if the assumption is not correct in all cases.

The first requirement in oral sustained release product is to establish the blood level of the drug at, or somewhat above, the level known to be therapeutically effective. The amount of drug needed to accomplish this is known for any drug prior to its incorporation into single-dose dosage forms. The second requirement is to maintain this level long enough to eliminate the necessity of additional dosage over a ten-to-twelve-hour period. Now, the maintenance of this level requires that drug is replaced as fast as it is eliminated. This requires that:

$$\text{Rate in} = \text{rate out} \qquad \text{(Eq. 1)}$$

For drugs that are eliminated by a first order process the rate out is equal to the rate of formation of degraded product, p,

$$dp/dt = Ka \qquad \text{(Eq. 2)}$$

where a is the amount present in the fluids of distribution at any time, t is time, and K the specific elimination constant. It is convenient to express K in terms of half-life in hours whence,

$$dp/dt = 0.693\ a/t^1/_2 \qquad \text{(Eq. 3)}$$

Substituting (3) in (1) yields

$$\text{Rate in} = 0.693\ a/t^1/_2 \qquad \text{(Eq. 4)}$$

The quantity, a, can be taken as the unit of weight that constituted the initial dose if the rate of establishment of initial blood level was comparatively rapid and no drug is lost at the absorption sites by reason of degradation reactions taking place there. If this initial dose resulted in a level of drug higher than necessary for therapeutic effect, a, can then be multiplied by some fraction, f, of the initial dose and equation 4 written

$$\text{Rate in} = 0.693\ b/t^1/_2 \qquad \text{(Eq. 5)}$$

where b is the product of a and f. The important point to recognize in Eq. 5 is that the quantity, b, is constant and input rate must be constant. The amount of drug, A, required to maintain level for some given number of hours, h, will be

$$A = \int_0^h 0.693\ b/t^1/_2\ dt = 0.693\ bh/t^1/_2 \qquad \text{(Eq. 6)}$$

Methods used to control the availability at the absorption site must release drug so that a plot of cumulative amount released with time is a straight line. This is in accord with a disclosure in a recent patent (3). The requirement of constant rate of release holds, regardless of the order of the elimination process from the fluids of distribution.

With drugs that undergo degradation at the absorption sites, Eq. 6 requires modification. If the reaction at the absorption site is also first order, Eq. 6 may be written:

$$A = 0.693\,[1/t^1/_2 + 1/t^d_{1/2}]\ bh \qquad \text{(Eq. 7)}$$

In Eq. 7, $t^d_{1/2}$ is the half-life in hours of the degradation reaction.

APPLICATION

Equation 6 has been used to calculate the data shown in Table I. The total amount of a drug included in a single dosage form would be the sum of the initial dose plus the maintenance dose. The maintenance dose would be released over the ten-hour period. Drugs that have a relatively short half-life and high initial dosage would be difficult to incorporate into a single dosage form from the formulation standpoint. Since maintenance dosage from Eq. 6 varies inversely with half-life, this problem could become difficult, even when

* Received January 29, 1957, from the School of Pharmacy, University of California Medical Center, San Francisco 22.

the initial dose is relatively small, if the half-life is very short. Data similar to those shown in Table I may be calculated for any drug, providing the half-life of drug in the fluids of distribution is known. The determination of this information under conditions of intravenous or oral administration has been recently reviewed (2).

TABLE I.—DOSAGE TO MAINTAIN CONSTANT BLOOD LEVEL FOR SEVERAL DRUGS OVER A TEN-HOUR PERIOD

Drug	Initial Dose (Gm.)	Half-life,[a] (Hrs.)	Level[b] of Peak, %	Maintenance Dosage (Gm.)
Sulfathiazole	1.00	4.0	70	1.21
Sulfapyridine	1.00	6.6	70	0.74
Theophylline	0.20	2.9	70	0.34
Sodium Salicylate	0.30	19.0	70	0.08
Tetracycline	0.25	8.0	70	0.15

[a] Half-lives in bloodstream data from reference (3).
[b] Equal to 100 × f in Eq. 6.

DISCUSSION

Equation 6 should hold in general, but several conditions may influence its application. The half-life must be the therapeutic half-life and if this does not correspond with that determined by measurement of blood concentration at various times, it must be estimated by some means.

Delay in absorption after solution should not cause much deviation in effects from application of Eq. 6. The result would be to cause leveling off of the concentration in the fluids of distribution at a value below that sought. The drop in level would be slight unless the absorption delay was excessively long. It is reasonable to suppose that absorption rate is concentration dependent and if concentration is maintained constant at the absorption sites, the rate should also be constant.

REFERENCES

(1) Edwards, L. J., *Trans. Faraday Soc.*, **47**, 1191(1951)
(2) Swintosky, J. V., THIS JOURNAL, **45**, 395(1956).
(3) U. S. pat. 2,738,303.

BOOKS RECEIVED

The Chemistry and Biology of Purines, Ciba Foundation Symposium. Edited by G. E. W. WOLSTENHOLME and CECILIA M. O'CONNOR. Little, Brown and Company, Boston, 1957. xii + 327 pp. 13.5 x 20.5 cm. Price $9.

International Encyclopedia of Cosmetic Material Trade Names. By MAISON G. DENAVARRE. Moore Publishing Co., New York, 1957. lvi + 369 pp. 15 x 23 cm. Price $7.50.

Experiments in Biochemical Research Techniques. By ROBERT W. COWGILL and ARTHUR B. PARDEE. John Wiley & Sons, Inc., New York, 1957. ix + 189 pp. 15 x 23 cm. Price $3.50.

Proceedings of the American Society for Testing Materials 1956. Vol. 56. American Society for Testing Materials, Philadelphia, Pa., 1957. xii + 1498 pp. 15 x 23 cm. Price $12.

Dialyse van Citraatbloed voor Transfusie (Dialysis of Citrated Blood for Transfusion—in Dutch, with summary in English). By Tekke Huizinga. Boekdrukkerij Voorheen Gebroeders Hoitsema, Groningen, Netherlands, 1957. 110 pp. 16.5 x 24 cm.

Actualities Pharmacologiques. By U. G. BIJLSMA, M. GUILLOT, *et al.* Edited by RENE HAZARD. Masson et Cie., Paris, 1956. 16.5 x 25 cm. 194 pp. Price 1,700 fr.

Basic Facts of Pharmacology. By S. M. BROOKS. W. B. Saunders Co., Philadelphia, Pa., 1957. 323 pp. 14 x 20.5 cm. Price $4.

Canadian Cancer Conference. Vol. II Edited by R. W. BEGG. Academic Press, Inc., New York 1957. xii + 398 pp. 15 x 33 cm. Price $8.50.

Exposés Annuels de Biochimie Médicale. 18th ed. Masson et Cie., Paris, 1957. 230 pp. 16.5 x 25 cm. Price 2,600 fr.

Man Against Germs. By A. L. BARON. E. P. Dutton & Co., Inc., New York, 1957. 320 pp. 13 x 20 cm. Price $4.50.

Médicaments de Synthèse. By H. P. KAUFMANN. Masson et Cie., Paris, 1957. vii + 853 pp. 16 x 25 cm. Price 11,500 fr.

The Chemistry of Plants. By ERSTON V. MILLER. Reinhold Publishing Corp., New York, 1957. vii + 174 pp. 15 x 23 cm. Price $4.75.

Clues to Suicide. Edited by EDWIN S. SHNEIDMAN and NORMAN L. FARBEROW. Foreword by Karl A. Menninger. McGraw-Hill Book Co., Inc., New York, 1957. xii + 227 pp. 14 x 21.5 cm. Price $5.50.

Health and Medical Care in New York City. A Report by The Committee for the Special Research Project in the Health Insurance Plan of Greater New York. Published for The Commonwealth Fund by the Harvard University Press, Cambridge, Mass., 1957. ix + 275 pp. 19.5 x 27 cm. Price $7.50.

Organic Reactions. Vol. IX. Edited by ROGER ADAMS. John Wiley & Sons, Inc., New York, 1957. vii + 468 pp. 15 x 23 cm. Price $12.

Phosphorus and Fluorine, The Chemistry and Toxic Action of Their Compounds. By BERNARD CHARLES SAUNDERS. New York: Cambridge University Press, New York, 1957. xiii + 231 pp. 14 x 21.5 cm. Price $5.

Solvent Extraction in Analytical Chemistry. By GEORGE H. MORRISON and HENRY FREISER. John Wiley & Sons, Inc., New York, 1957. xi + 269 pp. 15 x 23 cm. Price $6.75.

Surgeons All. By HARVEY GRAHAM. Philosophical Library, Inc., New York, 1957. xv + 459 pp. 15 x 23 cm. Price $10.

Apotekens Register över Standardförpackade Läkemedel 1957. Edited by the Apotekarsocietitens Laboratorieavdelning. Victor Pettersons Bokindustri Aktiebolag, Stockholm, 1957. xix + 481 pp. 17 x 23 cm.

British National Formulary 1957. (Standard Edition) Edited by the Joint Formulary Committee of the Pharmaceutical Society of Great Britian and the British Medical Association. The Pharmaceutical Press, London, 1957. 226 pp. 10.5 x 16.5 cm. Price 6*s*. (Interleaved copies 10*s*.)

Travaux des Laboratories de Matière Médicale et de Pharmacie Galénique de la Faculte de Pharmacie de Paris. Vol. XLI (1956). Edited by M. -M. JANOT and R. PARIS. Vigot Fréres, Éditeurs, Paris, 1957. 15.5 x 23.5 cm. 479 pp.

Principles de Synthèse Organique. Introduction au Méchanisme des Réactions. By JEAN MATHIEU and ANDRÉ ALLAIS with the collaboration of J. Valls and P. Poirier. Published under the direction of Léon Velluz. Masson et Cie., Éditeurs, Paris, 1957. x + 599 pp. 15 x 22 cm. Price 6,000 fr.

Practical Pharmacology. By J. H. BURN. Blackwell Scientific Publications, Oxford, 1957. vii + 72 pp. 18.5 x 24 cm. Price 12*s*. 6*d*. (net).

Rendiconti Istituto Superiore di Sanità. 1957 English Edition of Special Number on Pilot Plant Techniques of Submerged Fermentation. Vol. XVII (1954). Edited by Domenico Marotta and E. B. Chain. World Health Organization, 1957; Fondazione Emanuele Paternò, Rome, 1954. x + 243 pp. 17 x 24 cm. Price L. 5,000.

Subsidia Pharmaceutica I (S. Ph. I). Edited by Societe Suisse de Pharmacie and Le Commission de Centre Scientifique. Schweizerischer Apotheker-Verein, Zurich, 1957. 17 x 24.5 cm.

Pharmacopoea Internationalis. 1st ed. Vol. II. Translated into Japanese by Dr. Kiichiro Kakemi. World Health Organization Geneva 1957. vxi + 337 pp 15.5 x 24 cm.

BOOK NOTICES

A Guide to the Literature of Chemistry. 2nd ed. Edited by E. J. CRANE, AUSTIN M. PATTERSON, and ELEANOR B. MARR. John Wiley & Sons, Inc., New York, 1957. xiv + 395. 15 × 23 cm. Price $9.50.

It is noted that "no chemist can possibly know all of chemistry, but he can know how and where to look for chemical information." We can add that the authors of this guide are the best qualified individuals to supply the guideposts. The material is divided into chapters on: The problem and objectives, Books, Periodicals, Patents, Government publications, Trade literature, Other sources, Indexes, Libraries, and Procedure in literature searches. The appendix includes tabulated information and references to different phases of source material including an unusual listing of periodicals discontinued before 1910, and a particularly useful list of symbols and abbreviations used in the older chemical literature. Teachers in graduate courses and their students will find this volume of great assistance, as will anyone who is interested in this field.

Drug Resistance of Microorganisms. By ROBERT J. SCHNITZER and EMANUEL GRUNBERG. Academic Press Inc., New York, 1957. xiv + 395 pp. 15 × 23 cm. Price $10.

This book is a critical survey of the methods, fact, and theories regarding drug resistance and allied phenomena, e. g., therapeutic interference, and attempts to gather reported literature on experimental studies on protozoa, spirochetes, bacteria, and fungi. Much of the text is allotted to studies on the mechanism of drug resistance and the concluding portion deals with the theories of drug resistance. The 155 references utilized throughout the discussions are given at the end of the text, and author and subject indexes are appended. This excellently printed and bound volume deals with an increasingly important phase of bacteriology, mycology, and therapeutics. It should be available to students and investigators in these fields.

The Structure of Nucleic Acids and Their Role in Protein Synthesis. Biochemical Society Symposia No. 14. Edited by E. M. CROOK. New York: Cambridge University Press, 1957. 74 pp. 16 × 25 cm. Price $3.75.

This book is a compilation of the papers and discussions at the symposium held February 1956. The subjects presented are: Recent views on the chemical structure of the polynucleotides, Molecular structure of deoxyribose nucleic acid and nucleoprotein and possible implications in protein synthesis, Nucleic acids and protein synthesis in animal tissues, Nucleic acids and the incorporation of amino acids, and Interrelationships of nucleic acids and protein in the multiplications of bacteriophage.

For

Improved palatability

Alva Flavors, produced by successful specialists in the flavoring of medicinals, will effectively overcome most palatability problems confronting pharmaceutical manufacturers.

Alva produces both natural and artificial flavoring materials in conventional liquid form or in an hermetically sealed dry powder form called "Sealva flavors."

Suggestions, technical data and flavor samples will be submitted at your request.

VAN AMERINGEN-HAEBLER, INC.

521 WEST 57th STREET, NEW YORK 19, N. Y.

Out of Owens-Illinois' famed Technical Center comes . . .

KIMAX

bringing the unequalled Kimble reputation for quality, craftsmanship and accuracy in laboratory glassware to the medium of borosilicate glass.

KIMAX – Kimble's trade-mark for laboratory glassware made of tough, hard KG-33 borosilicate glass. Kimble thus becomes *your most complete source of laboratory glassware!*

KIMAX—the "hard glass" trade-mark assuring maximum accuracy, dependability, extra long life.

KIMAX—*for highest standards* of thermal and mechanical shock resistance...for outstanding resistance to chemical attack to assure a long life of sparkling clarity.

KIMAX—*ungraduated glassware* provides outstanding resistance to heat and mechanical shock and conforms to the most accurate tolerances of dimensional uniformity.

KIMAX – *graduated glassware* is individually retested for accuracy. Markings are permanent, stay sharp and clear for a lifetime.

KIMAX – *easy to repair* and modify by using simple glass blowing techniques. Can be sealed to and repaired with your present hard glassware...same coefficient of expansion.

KG-33 tubing is readily available for your special glass blowing needs.

KIMBLE—*Your most complete source of laboratory glassware* now provides your best opportunity for maximum quantity discounts!

Send for new Kimble Catalog now! The new Kimble Catalog offers you convenient, one-stop service for *all* your laboratory requirements. For your copy, write: Kimble Glass Company, subsidiary of Owens-Illinois, Dept. JA-9, Toledo 1, Ohio.

KIMAX LABORATORY GLASSWARE
AN (O) PRODUCT

OWENS-ILLINOIS
GENERAL OFFICES · TOLEDO 1, OHIO